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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 15/00

A2

(11) International Publication Number: WO 95/00554

(43) International Publication Date: 5 January 1995 (05.01.95)

(21) International Application Number: PCT/US94/06944

(22) International Filing Date: 17 June 1994 (17.06.94)

(30) Priority Data:
08/080,244 18 June 1993 (18.06.93) US
08/081,508 21 June 1993 (21.06.93) US
08/157,490 23 November 1993 (23.11.93) US

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(81) Designated States: AU, CA, FI, HU, JP, KR, NO, RO, RU, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS

(57) Abstract

Isolated mammalian nucleic acid molecules encoding receptor protein tyrosine kinases expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells are provided. Also included are the receptors encoded by such nucleic acid molecules; the nucleic acid molecules encoding receptor protein tyrosine kinases having the sequences shown in Figure 1a (murine Flk2), Figure 1b (human Flk2) and Figure 2 (murine Flk1); the receptor protein tyrosine kinases having the amino acid sequences shown in Figure 1a, Figure 1b and Figure 2; ligands for the receptors; nucleic acids sequences that encode the ligands; and methods of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

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TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS

This application is a continuation-in-part of serial number 08/125,669, filed September 23, 1993, which is a continuation-in-part of serial number 08/096,759, filed July 22, 1993, which is a continuation-in-part of serial number 08/081,508, filed June 21, 1993, which is a continuation-in-part of serial number 08/080,244, filed June 18, 1993, which is a continuation-in-part of serial number 08/076,022, filed June 9, 1993, which is a continuation-in-part of serial number 08/045,272, filed April 1, 1993, which is a continuation-in-part of serial number 08/005,941, filed January 15, 1993, which is a continuation-in-part of serial number 07/977,451, filed November 19, 1992, which is a continuation-in-part of serial number 07/975,049 filed November 12, 1992, which is a continuation-in-part of serial number 07/906,397 filed June 26, 1992 which is a continuation-in-part of serial number 07/813,593 filed December 24, 1991, which is a continuation-in-part of serial number 07/793,065 filed November 15, 1991, which is a continuation-in-part of serial number 07/728,913 filed June 28, 1991, which is a continuation-in-part of serial number 07/679,666 filed April 2, 1991, all of which are incorporated herein by reference.

The invention described in this application was made with U.S. government support from Grant Numbers R01-CA45339 and R01-DK42989 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to hematopoietic stem cell receptors, ligands for such receptors, and nucleic acid molecules encoding such receptors and ligands.

BACKGROUND OF THE INVENTION

The mammalian hematopoietic system comprises red and white blood cells. These cells are the mature cells that result from more primitive lineage-restricted cells. The cells of the hematopoietic system have been reviewed by Dexter and Spooncer in the Annual Review of Cell Biology 3, 423-441 (1987).

The red blood cells, or erythrocytes, result from primitive cells referred to by Dexter and Spooncer as erythroid burst-forming units (BFU-E). The immediate progeny of the erythroid burst-forming units are called erythroid colony-forming units (CFU-E).

The white blood cells contain the mature cells of the lymphoid and myeloid systems. The lymphoid cells include B lymphocytes and T lymphocytes. The B and T lymphocytes result from earlier progenitor cells referred to by Dexter and Spooncer as preT and preB cells.

The myeloid system comprises a number of cells including granulocytes, platelets, monocytes, macrophages, and megakaryocytes. The granulocytes are further divided into neutrophils, eosinophils, basophils and mast cells.

Each of the mature hematopoietic cells are specialized for specific functions. For example, erythrocytes are responsible for oxygen and carbon dioxide transport. T and B lymphocytes are responsible for cell-and antibody-mediated immune responses, respectively. Platelets are involved in blood clotting. Granulocytes and macrophages act generally as scavengers and accessory cells in the immune response against invading organisms and their by-products.

At the center of the hematopoietic system lie one or more

totipotent hematopoietic stem cells, which undergo a series of differentiation steps leading to increasingly lineage-restricted progenitor cells. The more mature progenitor cells are restricted to producing one or two lineages. Some examples of lineage-restricted progenitor cells mentioned by Dexter and Spooncer include granulocyte/macrophage colony-forming cells (GM-CFC), megakaryocyte colony-forming cells (Meg-CFC), eosinophil colony-forming cells (Eos-CFC), and basophil colony-forming cells (Bas-CFC). Other examples of progenitor cells are discussed above.

The hematopoietic system functions by means of a precisely controlled production of the various mature lineages. The totipotent stem cell possesses the ability both to self renew and to differentiate into committed progenitors for all hematopoietic lineages. These most primitive of hematopoietic cells are both necessary and sufficient for the complete and permanent hematopoietic reconstitution of a radiation-ablated hematopoietic system in mammals. The ability of stem cells to reconstitute the entire hematopoietic system is the basis of bone marrow transplant therapy.

It is known that growth factors play an important role in the development and operation of the mammalian hematopoietic system. The role of growth factors is complex, however, and not well understood at the present time. One reason for the uncertainty is that much of what is known about hematopoietic growth factors results from <u>in vitro</u> experiments. Such experiments do not necessarily reflect <u>in vivo</u> realities.

In addition, <u>in vitro</u> hematopoiesis can be established in the absence of added growth factors, provided that marrow stromal cells are added to the medium. The relationship between stromal cells and hematopoietic growth factors <u>in vivo</u> is not understood. Nevertheless, hematopoietic growth factors have been shown to be

highly active in vivo.

From what is known about them, hematopoietic growth factors appear to exhibit a spectrum of activities. At one end of the spectrum are growth factors such as erythropoietin, which is believed to promote proliferation only of mature erythroid progenitor cells. In the middle of the spectrum are growth factors such as IL-3, which is believed to facilitate the growth and development of early stem cells as well as of numerous progenitor cells. Some examples of progenitor cells induced by IL-3 include those restricted to the granulocyte/macrophage, eosinophil, megakaryocyte, erythroid and mast cell lineages.

At the other end of the spectrum is the hematopoietic growth factor that, along with the corresponding receptor, was discussed in a series of articles in the October 5, 1990 edition of Cell. The receptor is the product of the W locus, c-kit, which is a member of the class of receptor protein tyrosine kinases. The ligand for c-kit, which is referred to by various names such as stem cell factor (SCF) and mast cell growth factor (MGF), is believed to be essential for the development of early hematopoietic stem cells and cells restricted to the erythroid and mast cell lineages in mice; see, for example, Copeland et al., Cell 63, 175-183 (1990).

It appears, therefore, that there are growth factors that exclusively affect mature cells. There also appear to be growth factors that affect both mature cells and stem cells. The growth factors that affect both types of cells may affect a small number or a large number of mature cells.

There further appears to be an inverse relationship between the ability of a growth factor to affect mature cells and the ability of the growth factor to affect stem cells. For example, the c-kit ligand, which stimulates a small number of mature

cells, is believed to be more important in the renewal and development of stem cells then is IL-3, which is reported to stimulate proliferation of many mature cells (see above).

Prior to the present specification, there have been no reports of growth factors that exclusively stimulate stem cells in the absence of an effect on mature cells. The discovery of such growth factors would be of particular significance.

As mentioned above, c-kit is a protein tyrosine kinase (pTK). It is becoming increasingly apparent that the protein tyrosine kinases play an important role as cellular receptors for hematopoietic growth factors. Other receptor pTKs include the receptors of colony stimulating factor 1 (CSF-1) and PDGF.

The pTK family can be recognized by the presence of several conserved amino acid regions in the catalytic domain. These conserved regions are summarized by Hanks et al. in Science 241, 42-52 (1988), see Figure 1 starting on page 46 and by Wilks in Proc. Natl. Acad. Sci. USA 86, 1603-1607 (1989), see Figure 2 on page 1605.

Additional protein tyrosine kinases that represent hematopoietic growth factor receptors are needed in order more effectively to stimulate the self-renewal of the totipotent hematopoietic stem cell and to stimulate the development of all cells of the hematopoietic system both in vitro and in vivo. Novel hematopoietic growth factor receptors that are present only on primitive stem cells, but are not present on mature progenitor cells, are particularly desired. Ligands for the novel receptors are also desirable to act as hematopoietic growth factors. Nucleic acid sequences encoding the receptors and ligands are needed to produce recombinant receptors and ligands.

SUMMARY OF THE INVENTION

These and other objectives as will be apparent to those with ordinary skill in the art have been met by providing isolated mammalian nucleic acid molecules encoding receptor protein tyrosine kinases expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells. Also included are the receptors encoded by such nucleic acid molecules; the nucleic acid molecules encoding receptor protein tyrosine kinases having the sequences shown in Figure 1a.1-1a.6 (hereinafter Figure 1a)(murine Flk2), Figure 1b.1-1b.6 (hereinafter Figure 1b)(human Flk2) and Figure 2.1-2.9 (hereinafter Figure 2)(murine Flk1)(See SEQ. ID. NOS. 1, 3 and 5, respectively); the receptor protein tyrosine kinases having the amino acid sequences shown in Figure 1a, Figure 1b and Figure 2 (See SEQ. ID. NOS. 2, 4 and 6, respectively); ligands for the receptors; nucleic acid sequences that encode the ligands; and methods of stimulating the proliferation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

DESCRIPTION OF THE FIGURES

Figure 1a.1 through 1a.6 shows the cDNA and amino acid sequences of murine Flk2. All subsequent references to Figure 1a are intended to refer to Figure 1a.1 through 1a.6. The amino acid residues occur directly below the nucleotides in the open reading frame. Amino acids -27 to -1 constitute the hydrophobic leader sequence. Amino acids 1 to 517 constitute the extracellular receptor domain. Amino acids 518 to 537 constitute the transmembrane region. Amino acids 538 to 966 constitute the intracellular catalytic domain. Counting amino acid residue -27 as residue number 1, the following amino acid residues in the

intracellular domain are catalytic sub-domains identified by Hanks (see above): 618-623, 811-819, 832-834, 857-862, 872-878. The sequence at residues 709-785 is a signature sequence characteristic of Flk2. The protein tyrosine kinases generally have a signature sequence in this region. (See SEQ. ID. NOS. 1-2)

Figure 1b.1 through 1b.6 shows the complete cDNA and amino acid sequences of human Flk2 receptor. All subsequent references to Figure 1b are intended to refer to Figure 1b.1 through 1b.6. Amino acids -27 to -1 constitute the hydrophobic leader sequence. Amino acids 1 to 516 constitute the extracellular receptor domain. Amino acids 517 to 536 constitute the transmembrane region. Amino acids 537 to 966 constitute the intracellular catalytic domain. (See SEQ. ID. NOS. 3-4)

Figure 2.1 through 2.9 shows the cDNA and amino acid sequences of murine Flk1. All subsequent references to Figure 2 are intended to refer to Figure 2.1 through 2.9. Amino acids -19 to -1 constitute the hydrophobic leader sequence. Amino acids 1 to 743 constitute the extracellular receptor domain. Amino acids 744 to 765 constitute the transmembrane region. Amino acids 766 to 1348 constitute the intracellular catalytic domain. (See SEQ. ID. NOS. 5-6)

Figure 3 shows the time response of binding between a murine stromal cell line (2018) and APtag-Flk2 as well as APtag-Flk1. APtag without receptor (SEAP) is used as a control. See Example 8.

Figure 4 shows the dose response of binding between stromal cells (2018) and APtag-Flk2 as well as APtag-Flk1. APtag without receptor (SEAP) is used as a control. See Example 8.

DETAILED DESCRIPTION OF THE INVENTION

Receptors

In one embodiment, the invention relates to an isolated mammalian nucleic acid molecule encoding a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

The nucleic acid molecule may be a DNA, cDNA, or RNA molecule. The mammal in which the nucleic acid molecule exists may be any mammal, such as a mouse, rat, rabbit, or human.

The nucleic acid molecule encodes a protein tyrosine kinase (pTK). Members of the pTK family can be recognized by the conserved amino acid regions in the catalytic domains. Examples of pTK consensus sequences have been provided by Hanks et al. in Science 241, 42-52 (1988); see especially Figure 1 starting on page 46 and by Wilks in Proc. Natl. Acad. Sci. USA 86, 1603-1607 (1989); see especially Figure 2 on page 1605. A methionine residue at position 205 in the conserved sequence WMAPES is characteristic of pTK's that are receptors.

The Hanks et al article identifies eleven catalytic sub-domains containing pTK consensus residues and sequences. The pTKs of the present invention will have most or all of these consensus residues and sequences.

Some particularly strongly conserved residues and sequences are shown in Table 1.

TABLE 1

Conserved Residues and Sequences in pTKs[1]

Position[2]	Residue or Sequence	Catalytic Domain

50	G	I
52	G	I
57	V	I
70	A	II
72	K	II
91	E	III
166	D	VI
171	N	VI
184-186	DFG	VII
208	E	VIII
220	D	IX
225	G	IX
280	R	XI

1.See Hanks et al., Science 241, 42-52 (1988)
2.Adjusted in accordance with Hanks et al., Id.

A pTK of the invention may contain all thirteen of these highly conserved residues and sequences. As a result of natural or synthetic mutations, the pTKs of the invention may contain fewer than all thirteen strongly conserved residues and sequences, such as 11, 9, or 7 such sequences.

The receptors of the invention generally belong to the same class of pTK sequences that c-kit belongs to. It has surprisingly been discovered, however, that a new functional class of receptor pTKs exists. The new functional class of receptor pTKs is expressed in primitive hematopoietic cells, but not expressed in mature hematopoietic cells.

For the purpose of this specification, a primitive hematopoietic cell is totipotent, i.e. capable of reconstituting all hematopoietic blood cells *in vivo*. A mature hematopoietic cell is non-self-renewing, and has limited proliferative capacity - i.e., a limited ability to give rise to multiple lineages. Mature hematopoietic cells, for the purposes of this specification, are generally capable of giving rise to only one or two lineages *in vitro* or *in vivo*.

It should be understood that the hematopoietic system is complex, and contains many intermediate cells between the primitive totipotent hematopoietic stem cell and the totally committed mature hematopoietic cells defined above. As the stem cell develops into increasingly mature, lineage-restricted cells, it gradually loses its capacity for self-renewal.

The receptors of the present invention may and may not be expressed in these intermediate cells. The necessary and sufficient condition that defines members of the new class of receptors is that they are present in the primitive, totipotent stem cell or cells, and not in mature cells restricted only to one or, at most, two lineages.

An example of a member of the new class of receptor pTKs is called fetal liver kinase 2 (Flk2) after the organ in which it was found. There is approximately 1 totipotent stem cell per 10^4 cells in mid-gestation (day 14) fetal liver in mice. In addition to fetal liver, Flk2 is also expressed in fetal spleen, fetal thymus, adult brain, and adult marrow.

For example, Flk2 is expressed in individual multipotential CFU-Blast colonies capable of generating numerous multilineage colonies upon replating. It is likely, therefore, that Flk2 is expressed in the entire primitive (i.e. self-renewing) portion of the hematopoietic hierarchy. This discovery is consistent with Flk2 being important in transducing putative self-renewal signals from the environment.

It is particularly relevant that the expression of Flk2 mRNA occurs in the most primitive thymocyte subset. Even in two closely linked immature subsets that differ in expression of the IL-2 receptor, Flk2 expression segregates to the more primitive subset lacking an IL-2 receptor. The earliest thymocyte subset is believed to be uncommitted. Therefore, the thymocytes

expressing Flk2 may be multipotential. Flk2 is the first receptor tyrosine kinase known to be expressed in the T-lymphoid lineage.

The fetal liver mRNA migrates relative to 28S and 18S ribosomal bands on formaldehyde agarose gels at approximately 3.5 kb, while the brain message is considerably larger. In adult tissues, Flk2 m-RNA from both brain and bone marrow migrated at approximately 3.5 kb.

A second pTK receptor is also included in the present invention. This second receptor, which is called fetal liver kinase 1 (Flk1), is not a member of the same class of receptors as Flk2, since Flk1 may be found in some more mature hematopoietic cells. The amino acid sequence of murine Flk1 is given in Figure 2. (See SEQ. ID. NOS. 5-6)

The present invention includes the Flk1 receptor as well as DNA, cDNA and RNA encoding Flk1. The DNA sequence of murine Flk1 is also given in Figure 2. (See SEQ. ID. NO. 5) Flk1 may be found in the same organs as Flk2, as well as in fetal brain, stomach, kidney, lung, heart and intestine; and in adult kidney, heart, spleen, lung, muscle, and lymph nodes.

The receptor protein tyrosine kinases of the invention are known to be divided into easily found domains. The DNA sequence corresponding to the pTKs encode, starting at their 5'-ends, a hydrophobic leader sequence followed by a hydrophilic extracellular domain, which binds to, and is activated by, a specific ligand. Immediately downstream from the extracellular receptor domain, is a hydrophobic transmembrane region. The transmembrane region is immediately followed by a basic catalytic domain, which may easily be identified by reference to the Hanks et al. and Wilks articles discussed above.

The following table shows the nucleic acid and amino acid numbers that correspond to the signal peptide, the extracellular domain, the transmembrane region and the intracellular domain for murine Flk1 (mFlk1), murine Flk2 (mFlk2) and human Flk2 (hFlk2).

mFlk1

	Signal Peptide	Extracellular	Transmembrane	Intracellular
aa #	-19 to -1	1 to 743	744 to 765	766 to 1348
aa code	M A	A E	V V	R A
na #	208-264	265-2493	2494-2559	2560-4308

mFlk2

	Signal Peptide	Extracellular	Transmembrane	Intracellular
aa #	-27 to -1	1 to 517	518 to 537	538 to 966
aa code	M T	N S	F C	H S
na #	31-111	112-1662	1663-1722	1723-3006

hFlk2

	Signal Peptide	Extracellular	Transmembrane	Intracellular
aa #	-27 to -1	1 to 516	517 to 536	537 to 966
aa code	M N	Q F	Y C	H S
na #	58-138	139-1689	1690-1746	1747-3036

The present invention includes the extracellular receptor domain lacking the transmembrane region and catalytic domain. Preferably, the hydrophobic leader sequence is also removed from the extracellular domain. In the case of human and murine Flk2, the hydrophobic leader sequence includes amino acids -27 to -1. (See SEQ. ID. NOS. 2 and 4)

These regions and domains may easily be visually identified by those having ordinary skill in the art by reviewing the amino acid sequence in a suspected pTK and comparing it to known pTKs. For example, referring to Figure 1a, the transmembrane region of Flk2, which separates the extracellular receptor domain from the

catalytic domain, is encoded by nucleotides 1663 (T) to 1722 (C). These nucleotides correspond to amino acid residues 545 (Phe) to 564 (Cys). (See SEQ. ID. NOS. 1-2) The amino acid sequence between the transmembrane region and the catalytic sub-domain (amino acids 618-623) identified by Hanks et al. as sub-domain I (i.e., GXGXXG) is characteristic of receptor protein tyrosine kinases.

The extracellular domain may also be identified through commonly recognized criteria of extracellular amino acid sequences. The determination of appropriate criteria is known to those skilled in the art, and has been described, for example, by Hopp et al, Proc. Nat'l Acad. Sci. USA 78, 3824-3828 (1981); Kyte et al, J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol. 55, 836-839 (1985); Jameson et al, CA BIOS 4, 181-186 (1988); and Karplus et al, Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed characteristic of extracellular domains.

As will be discussed in more detail below, the nucleic acid molecules that encode the receptors of the invention may be inserted into known vectors for use in standard recombinant DNA techniques. Standard recombinant DNA techniques are those such as are described in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and by Ausubel et al., Eds, "Current Protocols in Molecular Biology," Green Publishing Associates and Wiley-Interscience, New York (1987). The vectors may be circular (i.e. plasmids) or non-circular. Standard vectors are available for cloning and expression in a host. The host may be prokaryotic or eucaryotic. Prokaryotic hosts are preferably E. coli. Preferred eucaryotic hosts include yeast, insect and mammalian cells. Preferred mammalian cells include, for example, CHO, COS and human cells.

Ligands

The invention also includes ligands that bind to the receptor pTKs of the invention. In addition to binding, the ligands stimulate the proliferation of additional primitive stem cells, differentiation into more mature progenitor cells, or both.

The ligand may be a growth factor that occurs naturally in a mammal, preferably the same mammal that produces the corresponding receptor. The growth factor may be isolated and purified, or be present on the surface of an isolated population of cells, such as stromal cells. A partial amino acid sequence of a Flk2 ligand is AQSLSFXFTKFDLD, wherein X is any amino acid. (See SEQ. ID. NO. 11)

The ligand may also be a molecule that does not occur naturally in a mammal. For example, antibodies, preferably monoclonal, raised against the receptors of the invention or against anti-ligand antibodies mimic the shape of, and act as, ligands if they constitute the negative image of the receptor or anti-ligand antibody binding site. The ligand may also be a non-protein molecule that acts as a ligand when it binds to, or otherwise comes into contact with, the receptor.

In another embodiment, nucleic acid molecules encoding the ligands of the invention are provided. The nucleic acid molecule may be RNA, DNA or cDNA.

Stimulating Proliferation of Stem Cells

The invention also includes a method of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells as defined above. The method comprises contacting the stem cells with a ligand in accordance with the

present invention. The stimulation of proliferation and/or differentiation may occur in vitro or in vivo.

The ability of a ligand according to the invention to stimulate proliferation of stem cells in vitro and in vivo has important therapeutic applications. Such applications include treating mammals, including humans, whose primitive stem cells do not sufficiently undergo self-renewal. Example of such medical problems include those that occur when defects in hematopoietic stem cells or their related growth factors depress the number of white blood cells. Examples of such medical problems include anemia, such as macrocytic and aplastic anemia. Bone marrow damage resulting from cancer chemotherapy and radiation is another example of a medical problem that would be helped by the stem cell factors of the invention.

Functional Equivalents

The invention includes functional equivalents of the pTK receptors, receptor domains, and ligands described above as well as of the nucleic acid sequences encoding them. A protein is considered a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has the same function as, the receptors and ligands of the invention. The equivalent may, for example, be a fragment of the protein, or a substitution, addition or deletion mutant of the protein.

For example, it is possible to substitute amino acids in a sequence with equivalent amino acids. Groups of amino acids known normally to be equivalent are:

(a)Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
(b)Asn(N) Asp(D) Glu(E) Gln(Q);
(c)His(H) Arg(R) Lys(K);

(d)Met(M) Leu(L) Ile(I) Val(V); and
(e)Phe(F) Tyr(Y) Trp(W).

Substitutions, additions and/or deletions in the receptors and ligands may be made as long as the resulting equivalent receptors and ligands are immunologically cross reactive with, and have the same function as, the native receptors and ligands.

The equivalent receptors and ligands will normally have substantially the same amino acid sequence as the native receptors and ligands. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions and/or deletions is considered to be an equivalent sequence. Preferably, less than 25%, more preferably less than 10%, and most preferably less than 5% of the number of amino acid residues in the amino acid sequence of the native receptors and ligands are substituted for, added to, or deleted from.

Equivalent nucleic acid molecules include nucleic acid sequences that encode equivalent receptors and ligands as defined above. Equivalent nucleic acid molecules also include nucleic acid sequences that differ from native nucleic acid sequences in ways that do not affect the corresponding amino acid sequences.

ISOLATION OF NUCLEIC ACID MOLECULES AND PROTEINS

Isolation of Nucleic Acid Molecules Encoding Receptors

In order to produce nucleic acid molecules encoding mammalian stem cell receptors, a source of stem cells is provided. Suitable sources include fetal liver, spleen, or thymus cells or adult marrow or brain cells.

For example, suitable mouse fetal liver cells may be

obtained at day 14 of gestation. Mouse fetal thymus cells may be obtained at day 14-18, preferably day 15, of gestation. Suitable fetal cells of other mammals are obtained at gestation times corresponding to those of mouse.

Total RNA is prepared by standard procedures from stem cell receptor-containing tissue. The total RNA is used to direct cDNA synthesis. Standard methods for isolating RNA and synthesizing cDNA are provided in standard manuals of molecular biology such as, for example, in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and in Ausubel et al., (Eds), "Current Protocols in Molecular Biology," Greene Associates/Wiley Interscience, New York (1990).

The cDNA of the receptors is amplified by known methods. For example, the cDNA may be used as a template for amplification by polymerase chain reaction (PCR); see Saiki et al., Science, 239, 487 (1988) or Mullis et al., U.S. patent 4,683,195. The sequences of the oligonucleotide primers for the PCR amplification are derived from the sequences of known receptors, such as from the sequences given in Figures 1a and 1b for Flk2 and in Figure 2 for Flk1, preferably from Flk2. (See SEQ. ID. NOS. 1, 3 and 5, respectively) The oligonucleotides are synthesized by methods known in the art. Suitable methods include those described by Caruthers in Science 230, 281-285 (1985).

In order to isolate the entire protein-coding regions for the receptors of the invention, the upstream oligonucleotide is complementary to the sequence at the 5′ end, preferably encompassing the ATG start codon and at least 5-10 nucleotides upstream of the start codon. The downstream oligonucleotide is complementary to the sequence at the 3′ end, optionally encompassing the stop codon. A mixture of upstream and downstream oligonucleotides are used in the PCR amplification.

The conditions are optimized for each particular primer pair according to standard procedures. The PCR product is analyzed by electrophoresis for the correct size cDNA corresponding to the sequence between the primers.

Alternatively, the coding region may be amplified in two or more overlapping fragments. The overlapping fragments are designed to include a restriction site permitting the assembly of the intact cDNA from the fragments.

The amplified DNA encoding the receptors of the invention may be replicated in a wide variety of cloning vectors in a wide variety of host cells. The host cell may be prokaryotic or eukaryotic. The DNA may be obtained from natural sources and, optionally, modified, or may be synthesized in whole or in part.

The vector into which the DNA is spliced may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13 and other filamentous single-stranded DNA phages.

Isolation of Receptors

DNA encoding the receptors of the invention are inserted into a suitable vector and expressed in a suitable prokaryotic or eucaryotic host. Vectors for expressing proteins in bacteria, especially E.coli, are known. Such vectors include the PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pEX); lambda P_L; maltose binding protein

(pMAL); and glutathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

Vectors useful in yeast are available. A suitable example is the 2μ plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters

of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

Vectors containing the receptor-encoding DNA and control signals are inserted into a host cell for expression of the receptor. Some useful expression host cells include well-known prokaryotic and eukaryotic cells. Some suitable prokaryotic hosts include, for example, E. coli, such as E. coli SG-936, E. coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli DHI, and E. coli MRCl, Pseudomonas, Bacillus, such as Bacillus subtilis, and Streptomyces.Suitable eukaryotic cells include yeast and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

The human homologs of the mouse receptors described above are isolated by a similar strategy. RNA encoding the receptors are obtained from a source of human cells enriched for primitive stem cells. Suitable human cells include fetal spleen, thymus and liver cells, and umbilical cord blood as well as adult brain and bone marrow cells. The human fetal cells are preferably obtained on the day of gestation corresponding to mid-gestation in mice. The amino acid sequences of the human flk receptors as well as of the nucleic acid sequences encoding them are homologous to the amino acid and nucleotide sequences of the mouse receptors.

In the present specification, the sequence of a first protein, such as a receptor or a ligand, or of a nucleic acid molecule that encodes the protein, is considered homologous to a second protein or nucleic acid molecule if the amino acid or nucleotide sequence of the first protein or nucleic acid molecule

is at least about 30% homologous, preferably at least about 50% homologous, and more preferably at least about 65% homologous to the respective sequences of the second protein or nucleic acid molecule. In the case of proteins having high homology, the amino acid or nucleotide sequence of the first protein or nucleic acid molecule is at least about 75% homologous, preferably at least about 85% homologous, and more preferably at least about 95% homologous to the amino acid or nucleotide sequence of the second protein or nucleic acid molecule.

Combinations of mouse oligonucleotide pairs are used as PCR primers to amplify the human homologs from the cells to account for sequence divergence. The remainder of the procedure for obtaining the human flk homologs are similar to those described above for obtaining mouse flk receptors. The less than perfect homology between the human flk homologs and the mouse oligonucleotides is taken into account in determining the stringency of the hybridization conditions.

Assay for expression of Receptors on Stem Cells

In order to demonstrate the expression of flk receptors on the surface of primitive hematopoietic stem cells, antibodies that recognize the receptor are raised. The receptor may be the entire protein as it exists in nature, or an antigenic fragment of the whole protein. Preferably, the fragment comprises the predicted extra-cellular portion of the molecule.

Antigenic fragments may be identified by methods known in the art. Fragments containing antigenic sequences may be selected on the basis of generally accepted criteria of potential antigenicity and/or exposure. Such criteria include the hydrophilicity and relative antigenic index, as determined by surface exposure analysis of proteins. The determination of appropriate criteria is known to those skilled in the art, and

has been described, for example, by Hopp et al, Proc. Nat'l Acad. Sci. USA 78, 3824-3828 (1981); Kyte et al, J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol. 55, 836-839 (1985); Jameson et al, CA BIOS 4, 181-186 (1988); and Karplus et al, Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed are selected preferentially over domains predicted to be more hydrophobic or hidden.

The proteins and fragments of the receptors to be used as antigens may be prepared by methods known in the art. Such methods include isolating or synthesizing DNA encoding the proteins and fragments, and using the DNA to produce recombinant proteins, as described above.

Fragments of proteins and DNA encoding the fragments may be chemically synthesized by methods known in the art from individual amino acids and nucleotides. Suitable methods for synthesizing protein fragments are described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984). Suitable methods for synthesizing DNA fragments are described by Caruthers in Science 230, 281-285 (1985).

If the receptor fragment defines the epitope, but is too short to be antigenic, it may be conjugated to a carrier molecule in order to produce antibodies. Some suitable carrier molecules include keyhole limpet hemocyanin, Ig sequences, TrpE, and human or bovine serum albumen. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule.

The antibodies are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These

methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al in Science 246, 1275-1281 (1989).

Polyclonal or monoclonal antisera shown to be reactive with receptor-encoded native proteins, such as with Flk1 and Flk2 encoded proteins, expressed on the surface of viable cells are used to isolate antibody-positive cells. One method for isolating such cells is flow cytometry; see, for example, Loken et al., European patent application 317,156. The cells obtained are assayed for stem cells by engraftment into radiation-ablated hosts by methods known in the art; see, for example, Jordan et al., Cell 61, 953-963 (1990).

Criteria for Novel Stem Cell Receptor Tyrosine Kinases Expressed in Stem Cells

Additional novel receptor tyrosine kinase cDNAs are obtained by amplifying cDNAs from stem cell populations using oligonucleotides as PCR primers; see above. Examples of suitable oligonucleotides are PTK1 and PTK2, which were described by Wilks et al. in Proc. Natl. Acad. Sci. USA 86, 1603-1607 (1989). Novel cDNA is selected on the basis of differential hybridization screening with probes representing known kinases. The cDNA clones hybridizing only at low stringency are selected and sequenced. The presence of the amino acid triplet DFG confirms that the sequence represents a kinase. The diagnostic methionine residue in the WMAPES motif is indicative of a receptor-like kinase, as described above. Potentially novel sequences obtained are compared to available sequences using databases such as

Genbank in order to confirm uniqueness. Gene-specific oligonucleotides are prepared as described above based on the sequence obtained. The oligonucleotides are used to analyze stem cell enriched and depleted populations for expression. Such cell populations in mice are described, for example, by Jordan et al. in Cell 61, 953-956 (1990); Ikuta et al. in Cell 62, 863-864 (1990); Spangrude et al. in Science 241, 58-62 (1988); and Szilvassy et al. in Blood 74, 930-939 (1989). Examples of such human cell populations are described as $CD33^{-}CD34^{+}$ by Andrews et al. in the Journal of Experimental Medicine 169, 1721-1731 (1989). Other human stem cell populations are described, for example, in Civin et al., European Patent Application 395,355 and in Loken et al., European Patent Application 317,156.

Isolating Ligands and Nucleic Acid Molecules Encoding Ligands

Cells that may be used for obtaining ligands include stromal cells, for example stromal cells from fetal liver, fetal spleen, fetal thymus and fetal or adult bone marrow. Cell lines expressing ligands are established and screened.

For example, cells such as stromal (non-hematopoietic) cells from fetal liver are immortalized by known methods. Examples of known methods of immortalizing cells include transduction with a temperature sensitive SV40 T-antigen expressed in a retroviral vector. Infection of fetal liver cells with this virus permits the rapid and efficient establishment of multiple independent cell lines. These lines are screened for ligand activity by methods known in the art, such as those outlined below.

Ligands for the receptors of the invention, such as Flk1 and Flk2, may be obtained from the cells in several ways. For example, a bioassay system for ligand activity employs chimeric tagged receptors; see, for example, Flanagan et al., Cell 63,

185-194 (1990). One strategy measures ligand binding directly via a histochemical assay. Fusion proteins comprising the extracellular receptor domains and secretable alkaline phosphatase (SEAP) are constructed and transfected into suitable cells such as NIH/3T3 or COS cells. Flanagan et al. refer to such DNA or amino acid constructs as APtag followed by the name of the receptor - i.e. APtag-c-kit. The fusion proteins bind with high affinity to cells expressing surface-bound ligand. Binding is detectable by the enzymatic activity of the alkaline phosphatase secreted into the medium. The bound cells, which are often stromal cells, are isolated from the APtag-receptor complex.

For example, some stromal cells that bind APtag-Flk1 and APtag-Flk2 fusion proteins include mouse fetal liver cells (see example 1); human fetal spleen cells (see example 3); and human fetal liver (example 3). Some stromal fetal thymus cells contain Flk1 ligand (example 3).

To clone the cDNA that encodes the ligand, a cDNA library is constructed from the isolated stromal cells in a suitable expression vector, preferably a phage such as CDM8, pSV Sport (BRL Gibco) or piH3, (Seed et al., Proc. Natl. Acad. Sci. USA 84, 3365-3369 (1987)). The library is transfected into suitable host cells, such as COS cells. Cells containing ligands on their surface are detected by known methods, see above.

In one such method, transfected COS cells are distributed into single cell suspensions and incubated with the secreted alkaline phosphatase-flk receptor fusion protein, which is present in the medium from NIH/3T3 or COS cells prepared by the method described by Flanagan et al., see above. Alkaline phosphatase-receptor fusion proteins that are not bound to the cells are removed by centrifugation, and the cells are panned on plates coated with antibodies to alkaline phosphatase. Bound

cells are isolated following several washes with a suitable wash reagent, such as 5% fetal bovine serum in PBS, and the DNA is extracted from the cells. Additional details of the panning method described above may be found in an article by Seed et al., Proc. Natl. Acad. Sci. USA 84, 3365-3369 (1987).

In a second strategy, the putative extracellular ligand binding domains of the receptors are fused to the transmembrane and kinase domains of the human c-fms tyrosine kinase and introduced into 3T3 fibroblasts. The human c-fms kinase is necessary and sufficient to transduce proliferative signals in these cells after appropriate activation i.e. with the Flk1 or Flk2 ligand. The 3T3 cells expressing the chimeras are used to screen putative sources of ligand in a cell proliferation assay.

An alternate approach for isolating ligands using the fusion receptor-expressing 3T3 cells and insertional activation is also possible. A retrovirus is introduced into random chromosomal positions in a large population of these cells. In a small fraction, the retrovirus is inserted in the vicinity of the ligand-encoding gene, thereby activating it. These cells proliferate due to autocrine stimulation of the receptor. The ligand gene is "tagged" by the retrovirus, thus facilitating its isolation.

Examples

Example 1. Cells containing mouse Flk1 and Flk2 ligands. Murine stromal cell line 2018.

In order to establish stromal cell lines, fetal liver cells are disaggregated with collagen and grown in a mixture of Dulbecco's Modified Eagle's Medium (DMEM) and 10% heat-inactivated fetal calf serum at 37°C. The cells are immortalized

by standard methods. A suitable method involves introducing DNA encoding a growth regulating- or oncogene-encoding sequence into the target host cell. The DNA may be introduced by means of transduction in a recombinant viral particle or transfection in a plasmid. See, for example, Hammerschmidt et al., Nature 340, 393-397 (1989) and Abcouwer et al, Biotechnology 7, 939-946 (1989). Retroviruses are the preferred viral vectors, although SV40 and Epstein-Barr virus can also serve as donors of the growth-enhancing sequences. A suitable retrovirus is the ecotropic retrovirus containing a temperature sensitive SV40 T-antigen (tsA58) and a G418 resistance gene described by McKay in Cell 66, 713-729 (1991). After several days at 37°C, the temperature of the medium is lowered to 32°C. Cells are selected with G418 (0.5 mg/ml). The selected cells are expanded and maintained.

A mouse stromal cell line produced by this procedure is called 2018 and was deposited on October 30, 1991 in the American Type Culture Collection, Rockville, Maryland, USA (ATCC); accession number CRL 10907.

Example 2. Cells containing human Flk1 and Flk2 ligands.

Human fetal liver (18, 20, and 33 weeks after abortion), spleen (18 weeks after abortion), or thymus (20 weeks after abortion) is removed at the time of abortion and stored on ice in a balanced salt solution. After mincing into 1 mm fragments and forcing through a wire mesh, the tissue is washed one time in Hanks Balanced Salt Solution (HBSS).

The disrupted tissue is centrifuged at 200 xg for 15 minutes at room temperature. The resulting pellet is resuspended in 10-20 ml of a tissue culture grade trypsin-EDTA solution (Flow Laboratories). The resuspended tissue is transferred to a

sterile flask and stirred with a stirring bar at room temperature for 10 minutes. One ml of heat-inactivated fetal bovine calf serum (Hyclone) is added to a final concentration of 10% in order to inhibit trypsin activity. Collagenase type IV (Sigma) is added from a stock solution (10 mg/ml in HBSS) to a final concentration of 100 ug/ml in order to disrupt the stromal cells. The tissue is stirred at room temperature for an additional 2.5 hours; collected by centrifugation (400xg, 15 minutes); and resuspended in "stromal medium," which contains Iscove's modification of DMEM supplemented with 10% heat-inactivated fetal calf serum, 5% heat-inactivated human serum (Sigma), 4 mM L-glutamine, 1x sodium pyruvate, (stock of 100x Sigma), 1x non-essential amino acids (stock of 100x, Flow), and a mixture of antibiotics kanomycin, neomycin, penicillin, streptomycin. Prior to resuspending the pellet in the stromal medium, the pellet is washed one time with HBSS. It is convenient to suspend the cells in 60 ml of medium. The number of cultures depends on the amount of tissue.

Example 3. Isolating Stromal cells

Resuspended Cells (example 2) that are incubated at 37°C with 5% carbon dioxide begin to adhere to the plastic plate within 10-48 hours. Confluent monolayers may be observed within 7-10 days, depending upon the number of cells plated in the initial innoculum. Non-adherent and highly refractile cells adhering to the stromal cell layer as colonies are separately removed by pipetting and frozen. Non-adherent cells are likely sources of populations of self-renewing stem cells containing Flk2. The adherent stromal cell layers are frozen in aliquots for future studies or expanded for growth in culture.

An unexpectedly high level of APtag-Flk2 fusion protein binding to the fetal spleen cells is observed. Two fetal spleen lines are grown in "stromal medium," which is described in

example 2.

Non-adherent fetal stem cells attach to the stromal cells and form colonies (colony forming unit - CFU). Stromal cells and CFU are isolated by means of sterile glass cylinders and expanded in culture. A clone, called Fsp 62891, contains the Flk2 ligand. Fsp 62891 was deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A on November 21, 1991, accession number CRL 10935.

Fetal liver and fetal thymus cells are prepared in a similar way. Both of these cell types produce ligands of Flk1 and, in the case of liver, some Flk2. One such fetal thymus cell line, called F.thy 62891, and one such fetal liver cell line, called FL 62891, were deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A on November 21, 1991 and April 2, 1992, respectively, accession numbers CRL 10936 and CRL 11005, respectively.

Stable human cell lines are prepared from fetal cells with the same temperature sensitive immortalizing virus used to prepare the murine cell line described in example 1.

Example 4. Isolation of human stromal cell clone

Highly refractile cells overgrow patches of stromal cells, presumably because the stromal cells produce factors that allow the formation of the CFU. To isolate stromal cell clones, sterile glass cylinders coated with vacuum grease are positioned over the CFU. A trypsin-EDTA solution (100 ml) is added in order to detach the cells. The cells are added to 5 ml of stromal medium and each (clone) plated in a single well of 6-well plate.

Example 5. Plasmid (AP-tag) for expressing secretable alkaline phosphatase (SEAP)

Plasmids that express secretable alkaline phosphatase are described by Flanagan and Leder in Cell 63, 185-194 (1990). The plasmids contain a promoter, such as the LTR promoter; a polylinker, including HindIII and BglII; DNA encoding SEAP; a poly-A signal; and ampicillin resistance gene; and replication site.

Example 6. Plasmid for expressing APtag-Flk2 and APtag-Flk1 fusion proteins

Plasmids that express fusion proteins of SEAP and the extracellular portion of either Flk1 or Flk2 are prepared in accordance with the protocols of Flanagan and Leader in Cell 63, 185-194 (1990) and Berger et al., Gene 66, 1-10 (1988). Briefly, a HindIII-Bam HI fragment containing the extracellular portion of Flk1 or Flk2 is prepared and inserted into the HindIII-BglII site of the plasmid described in example 5.

Example 7. Production Of APtag-Flk1 Or -Flk2 Fusion Protein

The plasmids from Example 6 are transfected into Cos-7 cells by DEAE-dextran (as described in Current Protocols in Molecular Biology, Unit 16.13, "Transient Expression of Proteins Using Cos Cells," 1991); and cotransfected with a selectable marker, such as pSV7neo, into NIH/3T3 cells by calcium precipitation. The NIH/3T3 cells are selected with 600μg/ml G418 in 100 mm plates. Over 300 clones are screened for secretion of placental alkaline phosphatase activity. The assay is performed by heating a portion of the supernatant at 65°C for 10 minutes to inactivate background phosphatase activity, and measuring the OD_{405} after incubating with 1M diethanolamine (pH 9.8), 0.5 mM $MgCl_2$, 10 mM L-homoarginine (a phosphatase inhibitor), 0.5 mg/ml BSA, and 12

mM p-nitrophenyl phosphate. Human placental alkaline phosphatase is used to perform a standard curve. The APtaq-Flk1 clones (F-1AP21-4) produce up to 10 μg alkaline phosphatase activity/ml and the APtaq-Flk2 clones (F-2AP26-0) produce up to 0.5 μg alkaline phosphatase activity/ml.

__Example 8. Assay For APtaq-Flk1 Or APtaq-Flk2 Binding To Cells__

The binding of APtaq-Flk1 or APtag-Flk2 to cells containing the appropriate ligand is assayed by standard methods. See, for example, Flanagan and Leder, Cell __63__:185-194, 1990). Cells (i.e., mouse stromal cells, human fetal liver, spleen or thymus, or various control cells) are grown to confluency in six-well plates and washed with HBHA (Hank's balanced salt solution with 0.5 mg/ml BSA, 0.02% NaN_3, 20 mM HEPES, pH 7.0). Supernatants from transfected COS or NIH/3T3 cells containing either APtaq-Flk1 fusion protein, APtag-Flk2 fusion protein, or APtag without a receptor (as a control) are added to the cell monolayers and incubated for two hours at room temperature on a rotating platform. The concentration of the APtaq-Flk1 fusion protein, APtag-Flk2 fusion protein, or APtag without a receptor is 60 ng/ml of alkaline phosphatase as determined by the standard alkaline phosphatase curve (see above). The cells are then rinsed seven times with HBHA and lysed in 350 μl of 1% Triton X-100, 10 mM Tris-HCl (pH 8.0). The lysates are transferred to a microfuge tube, along with a further 150 μl rinse with the same solution. After vortexing vigorously, the samples are centrifuged for five minutes in a microfuge, heated at 65°C for 12 minutes to inactivate cellular phosphatases, and assayed for phosphatase activity as described previously. Results of experiments designed to show the time and dose responses of binding between stromal cells containing the ligands to Flk2 and Flk1 (2018) and APtag-Flk2, APtag-Flk1 and APtag without receptor (as a control) are shown in Figures 3 and 4, respectively.

Example 8A. Plasmids for expressing Flk1/fms and Flk2/fms fusion proteins

Plasmids that express fusion proteins of the extracellular portion of either Flk1 or Flk2 and the intracellular portion of c-fms (also known as colony-stimulating factor-1 receptor) are prepared in a manner similar to that described under Example 6 (Plasmid for expressing APtag-Flk2 and APtag-Flk1 fusion proteins). Briefly, a Hind III - Bam HI fragment containing the extracellular portion of Flk1 or Flk2 is prepared and inserted into the Hind III - Bgl II site of a pLH expression vector containing the intracellular portion of c-fms.

8B. Expression of Flk1/fms or Flk2/fms in 3T3 cells

The plasmids from Example 8A are transfected into NIH/3T3 cells by calcium. The intracellular portion of c-fms is detected by Western blotting.

Example 9. Cloning and Expression of cDNA Coding For Mouse Ligand To Flk1 and Flk2 Receptors

cDNA expressing mouse ligand for Flk1 and Flk2 is prepared by known methods. See, for example, Seed, B., and Aruffo, A. PNAS 84:3365-3369, 1987; Simmons, D. and Seed, B. J. Immunol. 141:2797-2800; and D'Andrea, A.D., Lodish, H.F. and Wong, G.G. Cell 57:277-285, 1989).

The protocols are listed below in sequence: (a) RNA isolation; (b) poly A RNA preparation; (c) cDNA synthesis; (d) cDNA size fractionation; (e) propagation of plasmids (vector); (f) isolation of plasmid DNA; (g) preparation of vector pSV Sport (BRL Gibco) for cloning; (h) compilation of buffers for the above steps; (i) Transfection of cDNA encoding Ligands in Cos 7 Cells;

(j) panning procedure; (k) Expression cloning of Flk1 or Flk2 ligand by establishment of an autocrine loop.

9a. Guanidinium thiocyanate/LiCl Protocol for RNA Isolation

For each ml of mix desired, 0.5 g guanidine thiocyanate (GuSCN) is dissolved in 0.55 ml of 25% LiCl (stock filtered through 0.45 micron filter). 20 μl of mercaptoethanol is added. (The resulting solution is not good for more than about a week at room temperature.)

The 2018 stromal cells are centrifuged, and 1 ml of the solution described above is added to up to 5 x 10^7 cells. The cells are sheared by means of a polytron until the mixture is non-viscous. For small scale preparations (<10^8 cells), the sheared mixture is layered on 1.5 ml of 5.7M CsCl (RNase free; 1.26 g CsCl added to every ml 10 mM EDTA pH8), and overlaid with RNase-free water if needed. The mixture is spun in an SW55 rotor at 50 krpm for 2 hours. For large scale preparations, 25 ml of the mixture is layered on 12 ml CsCl in an SW28 tube, overlaid as above, and spun at 24 krpm for 8 hours. The contents of the tube are aspirated carefully with a sterile pasteur pipet connected to a vacuum flask. Once past the CsCl interface, a band around the tube is scratched with the pipet tip to prevent creeping of the layer on the wall down the tube. The remaining CsCl solution is aspirated. The resulting pellet is taken up in water, but not redissolved. 1/10 volume of sodium acetate and three volumes of ethanol are added to the mixture, and spun. The pellet is resuspended in water at 70°C, if necessary. The concentration of the RNA is adjusted to 1 mg/ml and frozen.

It should be noted that small RNA molecules (e.g., 5S) do not come down. For small amounts of cells, the volumes are scaled down, and the mixture is overlaid with GuSCN in RNase-free water on a gradient (precipitation is inefficient when RNA is

dilute).

9b. Poly A⁻ RNA preparation

(All buffers mentioned are compiled separately below)

A disposable polypropylene column is prepared by washing with 5M NaOH and then rinsing with RNase-free water. For each milligram of total RNA, approximately 0.3 ml (final packed bed) of oligo dT cellulose is added. The oligo dT cellulose is prepared by resuspending approximately 0.5 ml of dry powder in 1 ml of 0.1M NaOH and transferring it into the column, or by percolating 0.1M NaOH through a previously used column. The column is washed with several column volumes of RNase-free water until the pH is neutral, and rinsed with 2-3 ml of loading buffer. The column bed is transferred to a sterile 15 ml tube using 4-6 ml of loading buffer.

Total RNA from the 2018 cell line is heated to 70°C for 2-3 minutes. LiCl from RNase-free stock is added to the mixture to a final concentration of 0.5M. The mixture is combined with oligo dT cellulose in the 15 ml tube, which is vortexed or agitated for 10 minutes. The mixture is poured into the column, and washed with 3 ml loading buffer, and then with 3 ml of middle wash buffer. The mRNA is eluted directly into an SW55 tube with 1.5 ml of 2 mM EDTA and 0.1% SDS, discarding the first two or three drops.

The eluted mRNA is precipitated by adding 1/10 volume of 3M sodium acetate and filling the tube with ethanol. The contents of the tube are mixed, chilled for 30 minutes at -20°C, and spun at 50 krpm at 5°C for 30 minutes. After the ethanol is decanted, and the tube air dried, the mRNA pellet is resuspended in 50-100 µl of RNase-free water. 5 µl of the resuspended mRNA is heated to 70°C in MOPS/EDTA/formaldehyde, and examined on an RNase-free 1% agarose gel.

9c. cDNA Synthesis

The protocol used is a variation of the method described by Gubler and Hoffman in Gene 25, 263-270 (1983).

1. First Strand. 4 μg of mRNA is added to a microfuge tube, heated to approximately 100°C for 30 seconds, quenched on ice. The volume is adjusted to 70μl with RNAse-free water. 20 μl of RT1 buffer, 2 μl of RNAse inhibitor (Boehringer 36 u/μl), 1 μl of 5 μg/μl of oligo dT (Collaborative Research), 2.5 μl of 20 mM dXTP's (ultrapure - US Biochemicals), 1 μl of 1M DTT and 4 μl of RT-XL (Life Sciences, 24 u/μl) are added. The mixture is incubated at 42°C for 40 minutes, and inactivated by heating at 70°C for 10 minutes.

2. Second Strand. 320 μl of RNAse-free water, 80 μl of RT2 buffer, 5 μl of DNA Polymerase I (Boehringer, 5 U/μl), 2 μl RNAse H (BRL 2 u/μl) are added to the solution containing the first strand. The solution is incubated at 15°C for one hour and at 22°C for an additional hour. After adding 20 μl of 0.5M EDTA, pH 8.0, the solution is extracted with phenol and precipitated by adding NaCl to 0.5M linear polyacrylamide (carrier) to 20 μg/ml, and filling the tube with EtOH. The tube is spun for 2-3 minutes in a microfuge, vortexed to dislodge precipitated material from the wall of the tube, and respun for one minute.

3. Adaptors. Adaptors provide specific restriction sites to facilitate cloning, and are available from BRL Gibco, New England Biolabs, etc. Crude adaptors are resuspended at a concentration of 1 μg/μl. $MgSO_4$ is added to a final concentration of 10 mM, followed by five volumes of EtOH. The resulting precipitate is rinsed with 70% EtOH and resuspended in TE at a concentration of 1 μg/μl. To kinase, 25 μl of resuspended adaptors is added to 3 μl of 10X kinasing buffer and 20 units of kinase. The mixture is incubated at 37°C overnight. The precipitated cDNA is

resuspended in 240 μl of TE (10/1). After adding 30 μl of 10X low salt buffer, 30 μl of 10X ligation buffer with 0.1mM ATP, 3 μl (2.4 μg) of kinased 12-mer adaptor sequence, 2 μl (1.6 μg) of kinased 8-mer adaptor sequence, and 1 μl of T4 DNA ligase (BioLabs, 400 u/μl, or Boehringer, 1 Weiss unit ml), the mixture is incubated at 15°C overnight. The cDNA is extracted with phenol and precipitated as above, except that the extra carrier is omitted, and resuspended in 100 μl of TE.

9d. cDNA Size Fractionation.

A 20% KOAc, 2 mM EDTA, 1 μg/ml ethidium bromide solution and a 5% KOAc, 2 mM EDTA, 1 μg/ml ethidium bromide solution are prepared. 2.6 ml of the 20% KOAc solution is added to the back chamber of a small gradient maker. Air bubbles are removed from the tube connecting the two chambers by allowing the 20% solution to flow into the front chamber and forcing the solution to return to the back chamber by tilting the gradient maker. The passage between the chambers is closed, and 2.5 ml of 5% solution is added to the front chamber. Any liquid in the tubing from a previous run is removed by allowing the 5% solution to flow to the end of the tubing, and then to return to its chamber. The apparatus is placed on a stirplate, and, with rapid stirring, the topcock connecting the two chambers and the front stopcock are opened. A polyallomer 5W55 tube is filled from the bottom with the KOAc solution. The gradient is overlaid with 100 μl of cDNA solution, and spun for three hours at 50k rpm at 22°C. To collect fractions from the gradient, the SW55 tube is pierced close to the bottom of the tube with a butterfly infusion set (with the luer hub clipped off). Three 0.5 ml fractions and then six 0.25 ml fractions are collected in microfuge tubes (approximately 22 and 11 drops, respectively). The fractions are precipitated by adding linear polyacrylamide to 20 μg/ml and filling the tube to the top with ethanol. The tubes are cooled, spun in a microfuge tube for three minutes, vortexed, and respun

for one minute. The resulting pellets are rinsed with 70% ethanol and respun, taking care not to permit the pellets to dry to completion. Each 0.25 ml fraction is resuspended in 10 µl of TE, and 1 µl is run on a 1% agarose minigel. The first three fractions, and the last six which contain no material smaller than 1 kb are pooled.

9e. Propagation of Plasmids

SupF plasmids are selected in nonsuppressing bacterial hosts containing a second plasmid, p3, which contains amber mutated ampicillin and tetracycline drug resistance elements. See Seed, Nucleic Acids Res., 11, 2427-2445 (1983). The p3 plasmid is derived from RP1, is 57 kb in length, and is a stably maintained, single copy episome. The ampicillin resistance of this plasmid reverts at a high rate so that amp^r plasmids usually cannot be used in p3-containing strains. Selection for tetracycline resistance alone is almost as good as selection for ampicillin-tetracycline resistance. However, spontaneous appearance of chromosomal suppressor tRNA mutations presents an unavoidable background (frequency about 10^{-9}) in this system. Colonies arising from spontaneous suppressor mutations are usually larger than colonies arising from plasmid transformation. Suppressor plasmids are selected in Luria broth (LB) medium containing ampicillin at 12.5 µg/ml and tetracycline at 7.5 µg/ml. For scaled-up plasmid preparations, M9 Casamino acids medium containing glycerol (0.8%) is employed as a carbon source. The bacteria are grown to saturation.

Alternatively, pSV Sport (BRL, Gaithersberg, Maryland) may be employed to provide SV40 derived sequences for replication, transcription initiation and termination in COS 7 cells, as well as those sequences necessary for replication and ampicillin resistance in *E. coli*.

9f. Isolation of Vector DNA/Plasmid

One liter of saturated bacterial cells are spun down in J6 bottles at 4.2k rpm for 25 minutes. The cells are resuspended in 40 ml 10 mM EDTA, pH 8. 80 ml 0.2M NaOH and 1% SDS are added, and the mixture is swirled until it is clear and viscous. 40 ml 5M KOAc, pH 4.7 (2.5M KOAc, 2.5M HOAc) is added, and the mixture is shaken semi-vigorously until the lumps are approximately 2-3 mm in size. The bottle is spun at 4.2k rpm for 5 minutes. The supernatant is poured through cheesecloth into a 250 ml bottle, which is then filled with isopropyl alcohol and centrifuged at 4.2k rpm for 5 minutes. The bottle is gently drained and rinsed with 70% ethanol, taking care not to fragment the pellet. After inverting the bottle and removing traces of ethanol, the mixture is resuspended in 3.5 ml Tris base/EDTA (20 mM/10 mM). 3.75 ml of resuspended pellet and 0.75 ml 10 mg/ml ethidium bromide are added to 4.5 g CsCl. VTi80 tubes are filled with solution, and centrifuged for at least 2.5 hours at 80k rpm. Bands are extracted by visible light with 1 ml syringe and 20 gauge or lower needle. The top of the tube is cut off with scissors, and the needle is inserted upwards into the tube at an angle of about 30 degrees with respect to the tube at a position about 3 mm beneath the band, with the bevel of the needle up. After the band is removed, the contents of the tube are poured into bleach. The extracted band is deposited in a 13 ml Sarstedt tube, which is then filled to the top with n-butanol saturated with 1M NaCl extract. If the amount of DNA is large, the extraction procedure may be repeated. After aspirating the butanol into a trap containing 5M NaOH to destroy ethidium, an approximately equal volume of 1M ammonium acetate and approximately two volumes of 95% ethanol are added to the DNA, which is then spun at 10k rpm for 5 minutes. The pellet is rinsed carefully with 70% ethanol, and dried with a swab or lyophilizer.

9g. Preparation of Vector for Cloning

20 μg of vector is cut in a 200 μl reaction with 100 units of BstXI (New York Biolabs) at 50°C overnight in a well thermostated, circulating water bath. Potassium acetate solutions (5 and 20%) are prepared in 5W55 tubes as described above. 100 μl of the digested vector is added to each tube and spun for three hours, 50k rpm at 22°C. Under 300 nm UV light, the desired band is observed to migrate 2/3 of the length of the tube. Forward trailing of the band indicates that the gradient is overloaded. The band is removed with a 1 ml syringe fitted with a 20 gauge needle. After adding linear polyacrylamide and precipitating the plasmid by adding three volumes of ethanol, the plasmid is resuspended in 50 μl of TE. Trial ligations are carried out with a constant amount of vector and increasing amounts of cDNA. Large scale ligation are carried out on the basis of these trial ligations. Usually the entire cDNA prep requires 1-2 μg of cut vector.

9h. Buffers

Loading Buffer:.5M LiCl, 10 mM Tris pH 7.5, 1 mM EDTA .1% SDS.
Middle Wash Buffer:.15M LiCl, 10 mM Tris pH 7.5, 1 mM EDTA .1% SDS.
RT1 Buffer:.25M Tris pH 8.8 (8.2 at 42°), .25M KCl, 30 mM $MgCl_2$.
RT2 Buffer:.1M Tris pH 7.5, 25 mM MgCl2, .5M KCl, .25 mg/ml BSA, 50 mM dithiothreitol (DTT).
10X Low Salt:60 mM Tris pH 7.5, 60 mM $MgCl_2$, 50 mM NaCl, 2.5 mg/ml BSA 70 mM DME
10X Ligation Additions:1 mM ATP, 20 mM DTT, 1 mg/ml BSA 10 mM spermidine.
10X Kinasing Buffer:.5M Tris pH 7.5, 10 mM ATP, 20 mM DTT, 10 mM spermidine, 1 mg/ml BSA 100 mM MgCl2

9i. Transfection of cDNA encoding Ligands in Cos 7 Cells

Cos 7 cells are split 1:5 into 100 mm plates in Dulbecco's modified Eagles medium (DME)/10% fetal calf serum (FCS), and allowed to grow overnight. 3 ml Tris/DME (0.039M Tris, pH 7.4 in DME) containing 400 µg/ml DEAE-dextran (Sigma, D-9885) is prepared for each 100 mm plate of Cos 7 cells to be transfected. 10 µg of plasmid DNA preparation per plate is added. The medium is removed from the Cos-7 cells and the DNA/DEAE-dextran mixture is added. The cells are incubated for 4.5 hours. The medium is removed from the cells, and replaced with 3 ml of DME containing 2% fetal calf serum (FCS) and 0.1 mM chloroquine. The cells are incubated for one hour. After removing the chloroquine and replacing with 1.5 ml 20% glycerol in PBS, the cells are allowed to stand at room temperature for one minute. 3 ml Tris/DME is added, and the mixture is aspirated and washed two times with Tris/DME. 10 ml DME/10% FCS is added and the mixture is incubated overnight. The transfected Cos 7 cells are split 1:2 into fresh 100 mm plates with (DME)/10% FCS and allowed to grow.

9j. Panning Procedure for Cos 7 cells Expressing Ligand

1) Antibody-coated plates:

Bacteriological 100 mm plates are coated for 1.5 hours with rabbit anti-human placental alkaline phosphatase (Dako, California) diluted 1:500 in 10 ml of 50 mM Tris.HCl, pH 9.5. The plates are washed three times with 0.15M NaCl, and incubated with 3 mg BSA/ml PBS overnight. The blocking solution is aspirated, and the plates are utilized immediately or frozen for later use.

2) Panning cells:

The medium from transfected Cos 7 cells is aspirated, and 3 ml PBS/0.5 mM EDTA/0.02% sodium azide is added. The plates are incubated at 37°C for thirty minutes in order to detach the cells. The cells are triturated vigorously with a pasteur pipet and collected in a 15 ml centrifuge tube. The plate is washed with a further 2 ml PBS/EDTA/azide solution, which is then added to the centrifuge tube. After centrifuging at 200 xg for five minutes, the cells are resuspended in 3 ml of APtaq-Flk1 (F-1AP21-4) or Flk2 (F-2AP26-0) supernatant from transfected NIH/3T3 cells (see Example 7.), and incubated for 1.5 hours on ice. The cells are centrifuged again at 200 xg for five minutes. The supernatant is aspirated, and the cells are resuspended in 3 ml PBS/EDTA/azide solution. The cell suspension is layered carefully on 3 ml PBS/EDTA/azide/2% Ficoll, and centrifuged at 200 xg for four minutes. The supernatant is aspirated, and the cells are resuspended in 0.5 ml PBS/EDTA/azide solution. The cells are added to the antibody-coated plates containing 4 ml PBS/EDTA/azide/5% FBS, and allowed to stand at room temperature one to three hours. Non-adhering cells are removed by washing gently two or three times with 3 ml PBS/5% FBS.

3) Hirt Supernatant:

0.4 ml 0.6% SDS and 10 mM EDTA are added to the panned plates, which are allowed to stand 20 minutes. The viscous mixture is added by means of a pipet into a microfuge tube. 0.1 ml 5M NaCl is added to the tube, mixed, and chilled on ice for at least five hours. The tube is spun for four minutes, and the supernatant is removed carefully. The contents of the tube are extracted with phenol once, or, if the first interface is not clean, twice. Ten micrograms of linear polyacrylamide (or other carrier) is added, and the tube is filled to the top with ethanol. The resulting precipitate is resuspended in 0.1 ml

water or TE. After adding 3 volumes of EtOH/NaOAc, the cells are reprecipitated and resuspended in 0.1 ml water or TE. The cDNA obtained is transfected into any suitable E. coli host by electroporation. Suitable hosts are described in various catalogs, and include MC1061/p3 or Electromax DH10B Cells of BRL Gibco. The cDNA is extracted by conventional methods.

The above panning procedure is repeated until a pure E. coli clone bearing the cDNA as a unique plasmid recombinant capable of transfecting mammalian cells and yielding a positive panning assay is isolated. Normally, three repetitions are sufficient.

9k. Expression cloning of Flk1 or Flk2 ligand by establishment of an autocrine loop

Cells expressing Flk1/fms or Flk2/fms (Example 10) are transfected with 20-30 μg of a cDNA library from either Flk1 ligand or Flk2 ligand expressing stromal cells, respectively. The cDNA library is prepared as described above (a-h). The cells are co-transfected with 1 μg pLTR neo cDNA. Following transfection the cells are passaged 1:2 and cultured in 800 μg/ml of G418 in Dulbecco's medium (DME) supplemented with 10% CS. Approximately 12 days later the colonies of cells are passaged and plated onto dishes coated with poly -D- lysine (1 mg/ml) and human fibronectin (15 μg/ml). The culture medium is defined serum-free medium which is a mixture (3:1) of DME and Ham's F12 medium. The medium supplements are 8 mM $NaHCO_3$, 15 mM HEPES pH 7.4, 3 mM histidine, 4 μM $MnCl_2$, 10 uM ethanolamine, 0.1 μM selenous acid, 2 μM hydrocortisone, 5 μg/ml transferrin, 500 μg/ml bovine serum albumin/linoleic acid complex, and 20 μg/ml insulin (Ref. Zhan, X, et al. Oncogene 1: 369-376,1987). The cultures are refed the next day and every 3 days until the only cells capable of growing under the defined medium condition remain. The remaining colonies of cells are expanded and tested for the presence of the ligand by assaying for binding of APtag -

Flk1 or APtag - Flk2 to the cells (as described in Example 8). The DNA would be rescued from cells demonstrating the presence of the Flk1 or Flk2 ligand and the sequence.

Example 10. Expression of Ligand cDNA

The cDNA is sequenced, and expressed in a suitable host cell, such as a mammalian cell, preferably COS, CHO or NIH/3T3 cells. The presence of the ligand is confirmed by demonstrating binding of the ligand to APtag-Flk2 fusion protein (see above).

Example 11. Chemical Cross Linking of Receptor and Ligand

Cross linking experiments are performed on intact cells using a modification of the procedure described by Blume-Jensen et al et al., EMBO J., 10, 4121-4128 (1991). Cells are cultured in 100mm tissue culture plates to subconfluence and washed once with PBS-0.1% BSA.

To examine chemical cross linking of soluble receptor to membrane-bound ligand, stromal cells from the 2018 stromal cell line are incubated with conditioned media (CM) from transfected 3T3 cells expressing the soluble receptor Flk2-APtag. Cross linking studies of soluble ligand to membrane bound receptor are performed by incubating conditioned media from 2018 cells with transfected 3T3 cells expressing a Flk2-fms fusion construct.

Binding is carried out for 2 hours either at room temperature with CM containing 0.02% sodium azide to prevent receptor internalization or at 4°C with CM (and buffers) supplemented with sodium vanadate to prevent receptor dephosphorylation. Cells are washed twice with PBS-0.1% BSA and four times with PBS.

Cross linking is performed in PBS containing 250 mM

disuccinimidyl suberate (DSS; Pierce) for 30 minutes at room temperature. The reaction is quenched with Tris-HCL pH7.4 to a final concentration of 50 mM.

Cells are solubilized in solubilization buffer: 0.5% Triton - X100, 0.5% deoxycholic acid, 20 mM Tris pH 7.4, 150 mM NaCl, 10mM EDTA, 1mM PMFS, 50 mg/ml aprotinin, 2 mg/ml bestatin, 2 mg/ml pepstatin and 10mg/ml leupeptin. Lysed cells are immediately transferred to 1.5 ml Nalgene tubes and solubilized by rolling end to end for 45 minutes at 4°C. Lysates are then centrifuged in a microfuge at 14,000g for 10 minutes. Solubilized cross linked receptor complexes are then retrieved from lysates by incubating supernatants with 10% (v/v) wheat germ lectin-Sepharose 6MB beads (Pharmacia) at 4°C for 2 hours or overnight.

Beads are washed once with Tris-buffered saline (TBS) and resuspended in 2X SDS-polyacrylamide nonreducing sample buffer. Bound complexes are eluted from the beads by heating at 95°C for 5 minutes. Samples are analyzed on 4-12% gradient gels (NOVEX) under nonreducing and reducing conditions (0.35 M 2-mercaptoethanol) and then transferred to PVDF membranes for 2 hours using a Novex blotting apparatus. Blots are blocked in TBS-3% BSA for 1 hour at room temperature followed by incubation with appropriate antibody.

Cross linked Flk2-APtag and Flk2-fms receptors are detected using rabbit polyclonal antibodies raised against human alkaline phosphatase and fms protein, respectively. The remainder of the procedure is carried out according to the instructions provided in the ABC Kit (Pierce). The kit is based on the use of a biotinylated secondary antibody and avidin-biotinylated horseradish peroxidase complex for detection.

Example 12. Expression and purification of Flag-Flk2.

1. Design of the Flag-Flk2 expression plasmids.

A synthetic DNA fragment (Fragment 1) is synthesized using complementary oligonucleotides BP1 and BP2 (see below and SEQ. ID. NOS. 7 and 8). The fragment encoded the following features in the 5′ to 3′ order: Sal I restriction site, 22 base pair (bp) 5′ untranslated region containing an eukaryotic ribosome binding site, an ATG initiation codon, preprotrypsinogen signal sequence, coding region for the FLAG peptide (DYKDDDDKI) and Bgl II restriction site.

A cDNA fragment (Fragment 2) encoding Asn 27 to Ser 544 of murine Flk2 is obtained by polymerase chain reaction (PCR) using primers designed to introduce an in frame Bgl II site at the 5′ end (oligonucleotide BP5, see below and SEQ. ID. NO. 9) and a termination codon followed by a Not I site at the 3′ end (oligonucleotide BP10, see below and SEQ. ID. NO. 10). The template for the PCR reaction is full length Flk2 cDNA (Matthews et al., Cell 65:1143 (1991)). Fragment 2 is extensively digested with Bgl II and Not I restriction enzymes prior to ligation.

To assemble the complete Flag-Flk2 gene, Fragments 1 and 2 are ligated in a tripartate ligation into Sal I and Not I digested plasmid pSPORT (Gibco/BRL, Grand Island, NY) to give the plasmid pFlag-Flk2.

Preferably, the Flag-Flk2 protein is attached at either end to the Fc portion of an immunoglobulin (Ig). The Ig is preferably attached to the Flk2 portion of the Flag-Flk2 protein. To assemble the construct pFlag-Flk2-Ig, the sequences coding for the CH^1 domain of human immunoglobulin G (IgG^1) are placed downstream of the Flk2 coding region in the plasmid pFlag-Flk2 as per the method described by Zettlemeissl et al., DNA and Cell

Biology 9: 347-352 (1990).

The sequences of oligonucleotides used to construct the Flag-Flk2 gene are given below:

Oligonucleotide BP1:
5'-AATTCGTCGACTTTCTGTCACCATGAGTGCACTTCTGATCCTAGCCCTTGTG
GGAGCTGCTGTTGCTGACTACAAAGATGATGATGACAAGATCTA-3'

Oligonucleotide BP2:
5'-AGCTTAGATCTTGTCATCATCATCTTTGTAGTCAGCAACAGCAGCTCCCACA
AGGGCTAGGATCAGAAGTGCACTCATGGTGACAGAAAGTCGACG-3'

Oligonucleotide BP5:
5'-TGAGAAGATCTCAAACCAAGACCTGCCTGT-3'

Oligonucleotide BP10:
5'-CCAATGGCGGCCGCTCAGGAGATGTTGTCTTGGA-3'

(See SEQ. ID. NOS. 7-10, respectively)

2. Expression of the Flag-Flk2 construct.

For transient expression of the Flag-Flk2 construct, the Sall to Not I fragment from pFlag-Flk2 is subcloned into the plasmid pSVSPORT (Gibco/BRL) to give the plasmid pSVFlag-Flk2. For expression of the Flag-Flk2 protein pSVFlag-Flk2 is transfected into COS monkey cells using the DEAE-dextran method.

For stable expression in eukaryotic cells, the Sal I-Not I fragment of pFlag-Flk2 is cloned into the EcoRV and Not I sites of the plasmid pcDNA I/Neo (Invitrogen Co., San Diego, CA). The Sal I 3' recessed terminus of pFlag-Flk2 is filled with the Klenow fragment of DNA polymerase I and a mixture of

deoxyribonucleotides to make the site compatible with the EcoRV site of the vector. The resulting construct is introduced into cultured mammalian cells using either the Lipofectin (Gibco/BRL) or the calcium phosphate methods.

For expression in insect cells, the SalI to Hind III (from pSPORT polylinker) fragment of pFlag-Flk2 is subcloned into the BamH1-Hind III sites of the baculovirus transfer vector pBlueBac III (Invitrogen). The vector Bam HI site and the insert Sal I site are blunted with Klenow (see above). Production of the recombinant virus and infection of the Sf9 insect cells is performed as per manufacturers directions (Invitrogen).

Expression of the Flag-Flk2 protein is detected by Western blotting of SDS-PAGE separated conditioned media (mammalian cells) or cell lysates (insect cells) with the anti-Flag monoclonal antibody (mAb) M1 (International Biotechnology, Inc. [IBI], New Haven, CT).

3. Affinity purification of the Flag-Flk2 protein from conditioned media or insect cell lysates is performed using immobilized mAb M1 (IBI) as per manufacturers specifications.

3.1 Affinity purification of the Flag-Flk2-Ig[1] protein from conditioned media is performed using immobilized Protein A (Pharmacia LKB, Piscataway, NJ) as per the manufacturers instructions.

II. Use of the Flag-Flk2 protein to search for the Flk2 ligand.

1. Binding and cross-linking studies to detect membrane-bound ligand:

A. Binding studies.

Murine stromal lines (eg. 2018 cells ATCC CRL 10907 (see below), see example 1, *supra*) considered to be candidates for expression of the Flk2 ligand were deposited at the American Type Culture Collection, ATCC CRL 10907 (see below) and cultured in Dulbecco's modified Eagles medium (DMEM; Gibco/BRL) supplemented with 10% fetal calf serum. The cells are grown to confluency in 10 cm plates and washed once with PBS. Conditioned media containing Flag-Flk2 is incubated with the cells at 4°C for 2 hrs. The cell monolayers are rinsed extensively to remove the non-bound protein, solubilized and centrifuged to remove insoluble cellular material. Glycoproteins in the lysates are partially purified with wheat germ agglutinin-Sepharose (Pharmacia LKB, Piscataway, NJ), boiled in an SDS sample buffer, separated on SDS-PAGE gels and transferred to nitrocellulose membranes. The membranes are probed with the M1 antibody to detect the presence of cell-associated Flag-Flk2 protein.

B. In a cross-linking study, the above protocol is followed except that prior to solubilization the monolayer are treated with the crosslinker disuccinimidyl suberate (DSS; Pierce, Rockford, IL). The presence of a putative ligand is detected by an upward shift in the apparent molecular weight of the Flag-Flk2 band on Western blots.

C. Purified Flag-Flk2 protein labelled with Na125I via the Chloramine T method is used to asses the ability of the soluble extracellular domain of the Flk2 receptor to bind transmembrane form of the Flk2 ligand in cultured stromal lines. The labelled protein is added to monolayers of stromal cells on ice for 2 hr in the presence or absence of excess unlabelled protein. Specific binding is calculated by subtracting counts bound in the presence of excess unlabelled protein from the total counts bound.

2. Use of the Flag-Flk2 protein to search for secreted form of the ligand.

A. The Flag-Flk2 protein is used in attempts to identify the Flk2 ligand in conditioned media from stromal cell cultures via modification of the direct N-terminal sequencing method of Pan et al., Bioch. Biophys. Res. Comm. 166:201 (1990). Briefly, the Flag-Flk2 protein N-terminally sequenced by automatic Edman degradation chemistry an an ABI 477A sequncer with on line PTH amino acid analysis. Approximatelly 15 amino acids are determined. The protein is then immobilized on Nugel PAF silica beads via free NH4+ groups. The immobilized Flag-Flk2 is incubated with conditioned media from putative ligand-producing cells for 30 min at 4°C and washed free off non-bound proteins with phosphate buffered saline adjusted to 2M NaCl. The resulting protein complex is resequenced. For each sequencing cycle, any amino acid not expected at this position in the FLAG-Flk2 protein is considered as possibly originating from a protein complexed to the Flk2 receptor.

B. For conventional affinity chromatography, the Flag-Flk2 protein is immobilized on a stable support such as Sepharose. 35S-methionine labelled-conditioned media from stromal cell lines are passed over the affinity matrix and bound material is analyzed by SDS-PAGE gel electrophoresis and autoradiography.

3. Use of the Flag-Flk2 protein in expression cloning experiments.

A method of expression cloning of integral membrane proteins in COS cells has been described (Aruffo and Seed, Proc. Natl. Acad. Sci. 84:8573 (1987)). A cDNA library is prepared from an appropriate stromal cell line such as 2018 and is transfected into COS cells. Cells transiently expressing the Flk2 ligand are affinity adsorbed onto plastic plates coated with the Flag-Flk2 protein. The cells are lysed, the plasmid DNA is recovered and amplified in a bacterial host. The cycle of transfection into COS cells is repeated until a single cDNA clone encoding the ligand

molecule is isolated.

In a modification of the above technique, pools of transfected COS cells are screened for binding of 125I-Flag-Flk2. Positive cells pools are selected and plasmid DNA is recovered and amplified in E. coli. The resulting DNA preparation is used in subsequent rounds of transfection and transient expression until all cells are positive for binding of 125I-Flag-Flk2. The cDNA in the final plasmid preparation is then sequenced to determine the sequnce of the putative Flk-2 ligand.

Example 13 Isolating the Human Flk2 Ligand from PHA-LCM

13a. Source of the human Flk2 ligand

The Flk2 ligand is isolated from tissue culture medium conditioned by phytohemagglutinin-stimulated human peripheral blood leukocytes (PHA-LCM). The medium is prepared by isolating normal human peripheral blood mononuclear cells (leukocytes) from whole blood by density centrifugation (Ficoll-Hypaque, Pharmacia Biotech, Inc, Piscataway, NJ) and incubating these cells at a concentration of 2 X 10^6 cells/ml with the lectin phytohemagglutinin (PHA, Gibco Laboratories, Grand Island, NY) in a commercially-prepared, serum-free defined culture medium (AIMV; Gibco Laboratories, Grand Island, NY) for one week. PHA-LCM is harvested by removal of cells and debris by centrifugation.

13b. Isolating the human Flk2 ligand from PHA-LCM

The Flk2 ligand is one of a large number of proteins that are specifically secreted by PHA-activated cells into the medium. Several purification steps using conventional chromatographic techniques are required to isolate the Flk2 ligand. The chromatographic columns used (not listed in specific order) include: Blue Sepharose Fast Flow (Pharmacia Biotech, Inc,

Piscataway, NJ) to remove the medium component albumin, anion exchange (Q-Sepharose Fast Flow, Pharmacia Biotech, Inc, Piscataway, NJ) , cation exchange (S-Sepharose Fast Flow, Pharmacia Biotech, Inc, Piscataway, NJ), gel filtration (Superdex 75, Pharmacia Biotech, Inc, Piscataway, NJ), heparin sepharose (Pharmacia Biotech, Inc, Piscataway, NJ), ConA (Pharmacia Biotech, Inc, Piscataway, NJ), wheat germ agglutinin (Pharmacia Biotech, Inc, Piscataway, NJ), and C4 reverse phase (Vydac, The Separations Group, Hesperia, CA).

Biological assays are used throughout the purification to identify which column fractions contain the Flk2 ligand. The Flk2 ligand specifically stimulates proliferation *in vitro* of cell lines transfected with constructs expressing the full length Flk2 receptor or a chimeric receptor comprising of the the extracellular domain of the Flk2 receptor and the intracellular domain of a different protein tyrosine kinase receptor such as fms, the receptor for CSF-1. For example, the Flk2 ligand specifically stimulates proliferation of murine NIH 3T3 fibroblast cell line transfected with constructs expressing the murine or human Flk2 receptor in either full length or chimeric form (see example 8B). The parent untransfected 3T3 cells do not respond to the Flk2 ligand. The format of the Flk2 receptor 3T3 cell assay uses 96 well tissue culture plates (Becton Dickenson, Lincoln Park, NJ), where column fractions or other test samples are serially diluted across the plates in wells containing a mixture of AIMV and Dulbecco's modification of Eagle's medium (DMEM, Gibco Laboratories, Grand Island, NY). Samples are tested for their ability to stimulate proliferation of Flk2 receptor 3T3 cells initially cultured at 3×10^4 cells/well. Survival of Flk2 receptor 3T3 cells is dependent on the presence of the Flk2 ligand. Viable Flk2 receptor 3T3 cells are quantitated after three to five days in culture either visually or spectrophotometrically (Molecular Devices Corporation, Menlo Park, CA) using a tetraformazan salt (XTT,

Diagnostic Chemicals Ltd, Oxford, CT) that when cleaved by actively respiring cells forms diformazan salt which absorbs light at a wavelength (450 nm) that is different from the starting compound (560 nm). Relative (units/ml) and specific (units/mg) activities are defined as the reciprocal dilution at which half-maximal stimulation is detected.

13c. Physical properties of the human Flk2 ligand

The human Flk2 ligand isolated from PHA-LCM is a glycosylated protein and has an apparent molecular weight of 18 kDa, as determined by SDS-PAGE analysis run under reducing (β-mercaptoethanol) and non-reducing conditions. Its N-terminal fourteen amino acid sequence is A Q S L S F X F T K F D L D, wherein X is any amino acid. (See SEQ. ID. NO. 11) Its biological activity is inactivated at 100° C but not 60° C in five minutes and the activity is retained after the Flk2 ligand is subjected to a pH of 2.8 at room temperature for two hours.

The 18 kDa Flk2 ligand may act alone, in combination with other cytokines (e.g., interleukin 1, interleukin 3, interleukin 6, interleukin 11 or the kit ligand), or as a component of a complex of proteins that stimulate the Flk2 receptor in transfected 3T3 cell or in primitive hematopoietic progenitors. The complex of proteins may include a soluble or membrane-bound form of the Flk2 receptor.

A radiolabeled form of the Flk2 ligand may be used to detect and to measure the levels of Flk2 receptor, such as the soluble form of the Flk2 receptor, for example, in serum or urine of patients with bone marrow disorders.

13d. Biological activity of the human Flk2 ligand

In addition to acting on Flk2 receptor-expressing 3T3 cells,

the Flk2 ligand specifically stimulates proliferation of cells that naturally express the Flk2 receptor. In assays using either a human myeloid cell line or a subset of primitive hematopoietic progenitors expressing the surface phenotype CD34, the Flk2 ligand promotes proliferation but not differentiation into mature progeny. These observations suggest that the Flk2 ligand alone or in combination with other cytokines (e.g. Interleukin 1, Interleukin 3, Interleukin 6, Interleukin 11, or the kit ligand) may act to preserve or expand primitive hematopoietic progenitors *in vitro* and *in vivo*.

SUPPLEMENTAL ENABLEMENT

The invention as claimed is enabled in accordance with the above specification and readily available references and starting materials. Nevertheless, Applicants have deposited with the American Type Culture Collection, Rockville, Md., USA (ATCC) the cell lines listed below:

2018, ATCC accession no. CRL 10907, deposited October 30, 1991.

Fsp 62891, ATCC accession no. CRL 10935, deposited November 21, 1991.

F.thy 62891, ATCC accession no. CRL 10936, deposited November 21, 1991.

FL 62891, ATCC accession no. CRL 11005, deposited April 2, 1992.

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the regulations thereunder (Budapest Treaty). This assures

maintenance of a viable culture for 30 years from date of deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Applicants and ATCC which assures unrestricted availability upon issuance of the pertinent U.S. patent. Availability of the deposited strains is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lemischka, Ihor R.

(ii) TITLE OF INVENTION: TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS

(iii) NUMBER OF SEQUENCES: 11

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 23-SEP-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/679,666
(B) FILING DATE: 02-APR-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/728,913

(B) FILING DATE: 28-JUN-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/793,065
(B) FILING DATE: 15-NOV-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/813,593
(B) FILING DATE: 24-DEC-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/906,397
(B) FILING DATE: 26-JUN-1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/975,049
(B) FILING DATE: 12-NOV-1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/977,451
(B) FILING DATE: 19-NOV-1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/005,941
(B) FILING DATE: 15-JAN-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/045,272
(B) FILING DATE: 01-APR-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/076022
(B) FILING DATE: 09-JUN-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/080244
(B) FILING DATE: 18-JUN-1993

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(vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/081508
        (B) FILING DATE: 21-JUN-1993

(vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/096759
        (B) FILING DATE: 22-JUL-1993

(vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 08/125669
        (B) FILING DATE: 23-SEP-1993

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(2) INFORMATION FOR SEQ ID NO:1:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 3453 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: cDNA

 (iii) HYPOTHETICAL: NO

  (iv) ANTI-SENSE: NO

   (v) FRAGMENT TYPE: N-terminal
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(ix) FEATURE:
     (A) NAME/KEY: mat_peptide
     (B) LOCATION: 112..3006

(ix) FEATURE:
     (A) NAME/KEY: sig_peptide
     (B) LOCATION: 31..111

(ix) FEATURE:
     (A) NAME/KEY: CDS
     (B) LOCATION: 31..3009

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGGCCTGGC TACCGCGCGC TCCGGAGGCC ATG CGG GCG TTG GCG CAG CGC AGC         54
                                 Met Arg Ala Leu Ala Gln Arg Ser
                                 -27     -25                 -20

GAC CGG CGG CTG CTG CTG CTT GTT GTT TTG TCA GTA ATG ATT CTT GAG         102
Asp Arg Arg Leu Leu Leu Leu Val Val Leu Ser Val Met Ile Leu Glu
                -15                 -10                 -5

ACC GTT ACA AAC CAA GAC CTG CCT GTG ATC AAG TGT GTT TTA ATC AGT         150
Thr Val Thr Asn Gln Asp Leu Pro Val Ile Lys Cys Val Leu Ile Ser
             1                5                   10

CAT GAG AAC AAT GGC TCA TCA GCG GGA AAG CCA TCA TCG TAC CGA ATG         198
His Glu Asn Asn Gly Ser Ser Ala Gly Lys Pro Ser Ser Tyr Arg Met
     15                  20                  25

GTG CGA GGA TCC CCA GAA GAC CTC CAG TGT ACC CCG AGG CGC CAG AGT         246
Val Arg Gly Ser Pro Glu Asp Leu Gln Cys Thr Pro Arg Arg Gln Ser
 30                  35                  40                  45

GAA GGG ACG GTA TAT GAA GCG GCC ACC GTG GAG GTG GCC GAG TCT GGG         294
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58

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Glu Gly Thr Val Tyr Glu Ala Ala Thr Val Glu Val Ala Glu Ser Gly
                 50                  55                  60

TCC ATC ACC CTG CAA GTG CAG CTC GCC ACC CCA GGG GAC CTT TCC TGC      342
Ser Ile Thr Leu Gln Val Gln Leu Ala Thr Pro Gly Asp Leu Ser Cys
             65                  70                  75

CTC TGG GTC TTT AAG CAC AGC TCC CTG GGC TGC CAG CCG CAC TTT GAT      390
Leu Trp Val Phe Lys His Ser Ser Leu Gly Cys Gln Pro His Phe Asp
         80                  85                  90

TTA CAA AAC AGA GGA ATC GTT TCC ATG GCC ATC TTG AAC GTG ACA GAG      438
Leu Gln Asn Arg Gly Ile Val Ser Met Ala Ile Leu Asn Val Thr Glu
     95                 100                 105

ACC CAG GCA GGA GAA TAC CTA CTC CAT ATT CAG AGC GAA CGC GCC AAC      486
Thr Gln Ala Gly Glu Tyr Leu Leu His Ile Gln Ser Glu Arg Ala Asn
110                 115                 120                 125

TAC ACA GTA CTG TTC ACA GTG AAT GTA AGA GAT ACA CAG CTG TAT GTG      534
Tyr Thr Val Leu Phe Thr Val Asn Val Arg Asp Thr Gln Leu Tyr Val
                130                 135                 140

CTA AGG AGA CCT TAC TTT AGG AAG ATG GAA AAC CAG GAT GCA CTG CTC      582
Leu Arg Arg Pro Tyr Phe Arg Lys Met Glu Asn Gln Asp Ala Leu Leu
            145                 150                 155

TGC ATC TCC GAG GGT GTT CCG GAG CCC ACT GTG GAG TGG GTG CTC TGC      630
Cys Ile Ser Glu Gly Val Pro Glu Pro Thr Val Glu Trp Val Leu Cys
        160                 165                 170

AGC TCC CAC AGG GAA AGC TGT AAA GAA GAA GGC CCT GCT GTT GTC AGA      678
Ser Ser His Arg Glu Ser Cys Lys Glu Glu Gly Pro Ala Val Val Arg
    175                 180                 185

AAG GAG GAA AAG GTA CTT CAT GAG TTG TTC GGA ACA GAC ATC AGA TGC      726
Lys Glu Glu Lys Val Leu His Glu Leu Phe Gly Thr Asp Ile Arg Cys
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190                 195                 200                 205

TGT GCT AGA AAT GCA CTG GGC CGC GAA TGC ACC AAG CTG TTC ACC ATA       774
Cys Ala Arg Asn Ala Leu Gly Arg Glu Cys Thr Lys Leu Phe Thr Ile
                210                 215                 220

GAT CTA AAC CAG GCT CCT CAG AGC ACA CTG CCC CAG TTA TTC CTG AAA       822
Asp Leu Asn Gln Ala Pro Gln Ser Thr Leu Pro Gln Leu Phe Leu Lys
            225                 230                 235

GTG GGG GAA CCC TTG TGG ATC AGG TGT AAG GCC ATC CAT GTG AAC CAT       870
Val Gly Glu Pro Leu Trp Ile Arg Cys Lys Ala Ile His Val Asn His
        240                 245                 250

GGA TTC GGG CTC ACC TGG GAG CTG GAA GAC AAA GCC CTG GAG GAG GGC       918
Gly Phe Gly Leu Thr Trp Glu Leu Glu Asp Lys Ala Leu Glu Glu Gly
    255                 260                 265

AGC TAC TTT GAG ATG AGT ACC TAC TCC ACA AAC AGG ACC ATG ATT CGG       966
Ser Tyr Phe Glu Met Ser Thr Tyr Ser Thr Asn Arg Thr Met Ile Arg
270                 275                 280                 285

ATT CTC TTG GCC TTT GTG TCT TCC GTG GGA AGG AAC GAC ACC GGA TAT      1014
Ile Leu Leu Ala Phe Val Ser Ser Val Gly Arg Asn Asp Thr Gly Tyr
                290                 295                 300

TAC ACC TGC TCT TCC TCA AAG CAC CCC AGC CAG TCA GCG TTG GTG ACC      1062
Tyr Thr Cys Ser Ser Ser Lys His Pro Ser Gln Ser Ala Leu Val Thr
            305                 310                 315

ATC CTA GAA AAA GGG TTT ATA AAC GCT ACC AGC TCG CAA GAA GAG TAT      1110
Ile Leu Glu Lys Gly Phe Ile Asn Ala Thr Ser Ser Gln Glu Glu Tyr
        320                 325                 330

GAA ATT GAC CCG TAC GAA AAG TTC TGC TTC TCA GTC AGG TTT AAA GCG      1158
Glu Ile Asp Pro Tyr Glu Lys Phe Cys Phe Ser Val Arg Phe Lys Ala
    335                 340                 345
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TAC CCA CGA ATC CGA TGC ACG TGG ATC TTC TCT CAA GCC TCA TTT CCT     1206
Tyr Pro Arg Ile Arg Cys Thr Trp Ile Phe Ser Gln Ala Ser Phe Pro
350                 355                 360                 365

TGT GAA CAG AGA GGC CTG GAG GAT GGG TAC AGC ATA TCT AAA TTT TGC     1254
Cys Glu Gln Arg Gly Leu Glu Asp Gly Tyr Ser Ile Ser Lys Phe Cys
                370                 375                 380

GAT CAT AAG AAC AAG CCA GGA GAG TAC ATA TTC TAT GCA GAA AAT GAT     1302
Asp His Lys Asn Lys Pro Gly Glu Tyr Ile Phe Tyr Ala Glu Asn Asp
            385                 390                 395

GAC GCC CAG TTC ACC AAA ATG TTC ACG CTG AAT ATA AGA AAG AAA CCT     1350
Asp Ala Gln Phe Thr Lys Met Phe Thr Leu Asn Ile Arg Lys Lys Pro
        400                 405                 410

CAA GTG CTA GCA AAT GCC TCA GCC AGC CAG GCG TCC TGT TCC TCT GAT     1398
Gln Val Leu Ala Asn Ala Ser Ala Ser Gln Ala Ser Cys Ser Ser Asp
    415                 420                 425

GGC TAC CCG CTA CCC TCT TGG ACC TGG AAG AAG TGT TCG GAC AAA TCT     1446
Gly Tyr Pro Leu Pro Ser Trp Thr Trp Lys Lys Cys Ser Asp Lys Ser
430                 435                 440                 445

CCC AAT TGC ACG GAG GAA ATC CCA GAA GGA GTT TGG AAT AAA AAG GCT     1494
Pro Asn Cys Thr Glu Glu Ile Pro Glu Gly Val Trp Asn Lys Lys Ala
                450                 455                 460

AAC AGA AAA GTG TTT GGC CAG TGG GTG TCG AGC AGT ACT CTA AAT ATG     1542
Asn Arg Lys Val Phe Gly Gln Trp Val Ser Ser Ser Thr Leu Asn Met
            465                 470                 475

AGT GAG GCC GGG AAA GGG CTT CTG GTC AAA TGC TGT GCG TAC AAT TCT     1590
Ser Glu Ala Gly Lys Gly Leu Leu Val Lys Cys Cys Ala Tyr Asn Ser
        480                 485                 490

ATG GGC ACG TCT TGC GAA ACC ATC TTT TTA AAC TCA CCA GGC CCC TTC     1638
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Met Gly Thr Ser Cys Glu Thr Ile Phe Leu Asn Ser Pro Gly Pro Phe
    495                 500                 505

CCT TTC ATC CAA GAC AAC ATC TCC TTC TAT GCG ACC ATT GGG CTC TGT      1686
Pro Phe Ile Gln Asp Asn Ile Ser Phe Tyr Ala Thr Ile Gly Leu Cys
510                 515                 520                 525

CTC CCC TTC ATT GTT GTT CTC ATT GTG TTG ATC TGC CAC AAA TAC AAA      1734
Leu Pro Phe Ile Val Val Leu Ile Val Leu Ile Cys His Lys Tyr Lys
                530                 535                 540

AAG CAA TTT AGG TAC GAG AGT CAG CTG CAG ATG ATC CAG GTG ACT GGC      1782
Lys Gln Phe Arg Tyr Glu Ser Gln Leu Gln Met Ile Gln Val Thr Gly
            545                 550                 555

CCC CTG GAT AAC GAG TAC TTC TAC GTT GAC TTC AGG GAC TAT GAA TAT      1830
Pro Leu Asp Asn Glu Tyr Phe Tyr Val Asp Phe Arg Asp Tyr Glu Tyr
        560                 565                 570

GAC CTT AAG TGG GAG TTC CCG AGA GAG AAC TTA GAG TTT GGG AAG GTC      1878
Asp Leu Lys Trp Glu Phe Pro Arg Glu Asn Leu Glu Phe Gly Lys Val
    575                 580                 585

CTG GGG TCT GGC GCT TTC GGG AGG GTG ATG AAC GCC ACG GCC TAT GGC      1926
Leu Gly Ser Gly Ala Phe Gly Arg Val Met Asn Ala Thr Ala Tyr Gly
590                 595                 600                 605

ATT AGT AAA ACG GGA GTC TCA ATT CAG GTG GCG GTG AAG ATG CTA AAA      1974
Ile Ser Lys Thr Gly Val Ser Ile Gln Val Ala Val Lys Met Leu Lys
                610                 615                 620

GAG AAA GCT GAC AGC TGT GAA AAA GAA GCT CTC ATG TCG GAG CTC AAA      2022
Glu Lys Ala Asp Ser Cys Glu Lys Glu Ala Leu Met Ser Glu Leu Lys
            625                 630                 635

ATG ATG ACC CAC CTG GGA CAC CAT GAC AAC ATC GTG AAT CTG CTG GGG      2070
Met Met Thr His Leu Gly His His Asp Asn Ile Val Asn Leu Leu Gly
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            640                                645                                650

GCA TGC ACA CTG TCA GGG CCA GTG TAC TTG ATT TTT GAA TAT TGT TGC        2118
Ala Cys Thr Leu Ser Gly Pro Val Tyr Leu Ile Phe Glu Tyr Cys Cys
    655                 660                 665

TAT GGT GAC CTC CTC AAC TAC CTA AGA AGT AAA AGA GAG AAG TTT CAC        2166
Tyr Gly Asp Leu Leu Asn Tyr Leu Arg Ser Lys Arg Glu Lys Phe His
670                 675                 680                 685

AGG ACA TGG ACA GAG ATT TTT AAG GAA CAT AAT TTC AGT TCT TAC CCT        2214
Arg Thr Trp Thr Glu Ile Phe Lys Glu His Asn Phe Ser Ser Tyr Pro
                690                 695                 700

ACT TTC CAG GCA CAT TCA AAT TCC AGC ATG CCT GGT TCA CGA GAA GTT        2262
Thr Phe Gln Ala His Ser Asn Ser Ser Met Pro Gly Ser Arg Glu Val
            705                 710                 715

CAG TTA CAC CCG CCC TTG GAT CAG CTC TCA GGG TTC AAT GGG AAT TCA        2310
Gln Leu His Pro Pro Leu Asp Gln Leu Ser Gly Phe Asn Gly Asn Ser
        720                 725                 730

ATT CAT TCT GAA GAT GAG ATT GAA TAT GAA AAC CAG AAG AGG CTG GCA        2358
Ile His Ser Glu Asp Glu Ile Glu Tyr Glu Asn Gln Lys Arg Leu Ala
    735                 740                 745

GAA GAA GAG GAG GAA GAT TTG AAC GTG CTG ACG TTT GAA GAC CTC CTT        2406
Glu Glu Glu Glu Glu Asp Leu Asn Val Leu Thr Phe Glu Asp Leu Leu
750                 755                 760                 765

TGC TTT GCG TAC CAA GTG GCC AAA GGC ATG GAA TTC CTG GAG TTC AAG        2454
Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu Phe Leu Glu Phe Lys
                770                 775                 780

TCG TGT GTC CAC AGA GAC CTG GCA GCC AGG AAT GTG TTG GTC ACC CAC        2502
Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr His
            785                 790                 795
```

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GGG AAG GTG GTG AAG ATC TGT GAC TTT GGA CTG GCC CGA GAC ATC CTG      2550
Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Leu
        800                 805                 810

AGC GAC TCC AGC TAC GTC GTC AGG GGC AAC GCA CGG CTG CCG GTG AAG      2598
Ser Asp Ser Ser Tyr Val Val Arg Gly Asn Ala Arg Leu Pro Val Lys
    815                 820                 825

TGG ATG GCA CCC GAG AGC TTA TTT GAA GGG ATC TAC ACA ATC AAG AGT      2646
Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile Tyr Thr Ile Lys Ser
830                 835                 840                 845

GAC GTC TGG TCC TAC GGC ATC CTT CTC TGG GAG ATA TTT TCA CTG GGT      2694
Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu Gly
                850                 855                 860

GTG AAC CCT TAC CCT GGC ATT CCT GTC GAC GCT AAC TTC TAT AAA CTG      2742
Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala Asn Phe Tyr Lys Leu
            865                 870                 875

ATT CAG AGT GGA TTT AAA ATG GAG CAG CCA TTC TAT GCC ACA GAA GGG      2790
Ile Gln Ser Gly Phe Lys Met Glu Gln Pro Phe Tyr Ala Thr Glu Gly
        880                 885                 890

ATA TAC TTT GTA ATG CAA TCC TGC TGG GCT TTT GAC TCA AGG AAG CGG      2838
Ile Tyr Phe Val Met Gln Ser Cys Trp Ala Phe Asp Ser Arg Lys Arg
    895                 900                 905

CCA TCC TTC CCC AAC CTG ACT TCA TTT TTA GGA TGT CAG CTG GCA GAG      2886
Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly Cys Gln Leu Ala Glu
910                 915                 920                 925

GCA GAA GAA GCA TGT ATC AGA ACA TCC ATC CAT CTA CCA AAA CAG GCG      2934
Ala Glu Glu Ala Cys Ile Arg Thr Ser Ile His Leu Pro Lys Gln Ala
                930                 935                 940

GCC CCT CAG CAG AGA GGC GGG CTC AGA GCC CAG TCG CCA CAG CGC CAG      2982
```

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Ala Pro Gln Gln Arg Gly Gly Leu Arg Ala Gln Ser Pro Gln Arg Gln
            945                     950                 955

GTG AAG ATT CAC AGA GAA AGA AGT TAGCGAGGAG GCCTTGGACC CCGCCACCCT    3036
Val Lys Ile His Arg Glu Arg Ser
        960                 965

AGCAGGCTGT AGACCGCAGA GCCAAGATTA GCCTCGCCTC TGAGGAAGCG CCCTACAGCG   3096

CGTTGCTTCG CTGGACTTTT CTCTAGATGC TGTCTGCCAT TACTCCAAAG TGACTTCTAT   3156

AAAATCAAAC CTCTCCTCGC ACAGGCGGGA GAGCCAATAA TGAGACTTGT TGGTGAGCCC   3216

GCCTACCCTG GGGGCCTTTC CACGAGCTTG AGGGGAAAGC CATGTATCTG AAATATAGTA   3276

TATTCTTGTA AATACGTGAA ACAAACCAAA CCCGTTTTTT GCTAAGGGAA AGCTAAATAT   3336

GATTTTTAAA AATCTATGTT TTAAAATACT ATGTAACTTT TTCATCTATT TAGTGATATA   3396

TTTTATGGAT GGAAATAAAC TTTCTACTGT AAAAAAAAAA AAAAAAAAAA AAAAAAA      3453
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 992 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Arg Ala Leu Ala Gln Arg Ser Asp Arg Arg Leu Leu Leu Leu Val
-27     -25                 -20                 -15

Val Leu Ser Val Met Ile Leu Glu Thr Val Thr Asn Gln Asp Leu Pro
```

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    -10                      -5                       1                   5

Val Ile Lys Cys Val Leu Ile Ser His Glu Asn Asn Gly Ser Ser Ala
                 10                  15                  20

Gly Lys Pro Ser Ser Tyr Arg Met Val Arg Gly Ser Pro Glu Asp Leu
             25                  30                  35

Gln Cys Thr Pro Arg Arg Gln Ser Glu Gly Thr Val Tyr Glu Ala Ala
         40                  45                  50

Thr Val Glu Val Ala Glu Ser Gly Ser Ile Thr Leu Gln Val Gln Leu
     55                  60                  65

Ala Thr Pro Gly Asp Leu Ser Cys Leu Trp Val Phe Lys His Ser Ser
 70                  75                  80                  85

Leu Gly Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Ile Val Ser
                 90                  95                 100

Met Ala Ile Leu Asn Val Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu
            105                 110                 115

His Ile Gln Ser Glu Arg Ala Asn Tyr Thr Val Leu Phe Thr Val Asn
        120                 125                 130

Val Arg Asp Thr Gln Leu Tyr Val Leu Arg Arg Pro Tyr Phe Arg Lys
    135                 140                 145

Met Glu Asn Gln Asp Ala Leu Leu Cys Ile Ser Glu Gly Val Pro Glu
150                 155                 160                 165

Pro Thr Val Glu Trp Val Leu Cys Ser Ser His Arg Glu Ser Cys Lys
                170                 175                 180

Glu Glu Gly Pro Ala Val Val Arg Lys Glu Glu Lys Val Leu His Glu
            185                 190                 195
```

```
Leu Phe Gly Thr Asp Ile Arg Cys Cys Ala Arg Asn Ala Leu Gly Arg
        200                 205                 210

Glu Cys Thr Lys Leu Phe Thr Ile Asp Leu Asn Gln Ala Pro Gln Ser
    215                 220                 225

Thr Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg
230                 235                 240                 245

Cys Lys Ala Ile His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu
                250                 255                 260

Glu Asp Lys Ala Leu Glu Glu Gly Ser Tyr Phe Glu Met Ser Thr Tyr
            265                 270                 275

Ser Thr Asn Arg Thr Met Ile Arg Ile Leu Leu Ala Phe Val Ser Ser
        280                 285                 290

Val Gly Arg Asn Asp Thr Gly Tyr Tyr Thr Cys Ser Ser Ser Lys His
    295                 300                 305

Pro Ser Gln Ser Ala Leu Val Thr Ile Leu Glu Lys Gly Phe Ile Asn
310                 315                 320                 325

Ala Thr Ser Ser Gln Glu Glu Tyr Glu Ile Asp Pro Tyr Glu Lys Phe
                330                 335                 340

Cys Phe Ser Val Arg Phe Lys Ala Tyr Pro Arg Ile Arg Cys Thr Trp
            345                 350                 355

Ile Phe Ser Gln Ala Ser Phe Pro Cys Glu Gln Arg Gly Leu Glu Asp
        360                 365                 370

Gly Tyr Ser Ile Ser Lys Phe Cys Asp His Lys Asn Lys Pro Gly Glu
    375                 380                 385

Tyr Ile Phe Tyr Ala Glu Asn Asp Asp Ala Gln Phe Thr Lys Met Phe
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390                 395                 400                 405
Thr Leu Asn Ile Arg Lys Lys Pro Gln Val Leu Ala Asn Ala Ser Ala
                410                 415                 420

Ser Gln Ala Ser Cys Ser Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr
            425                 430                 435

Trp Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Pro
        440                 445                 450

Glu Gly Val Trp Asn Lys Lys Ala Asn Arg Lys Val Phe Gly Gln Trp
    455                 460                 465

Val Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Gly Lys Gly Leu Leu
470                 475                 480                 485

Val Lys Cys Cys Ala Tyr Asn Ser Met Gly Thr Ser Cys Glu Thr Ile
                490                 495                 500

Phe Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser
            505                 510                 515

Phe Tyr Ala Thr Ile Gly Leu Cys Leu Pro Phe Ile Val Val Leu Ile
        520                 525                 530

Val Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln
    535                 540                 545

Leu Gln Met Ile Gln Val Thr Gly Pro Leu Asp Asn Glu Tyr Phe Tyr
550                 555                 560                 565

Val Asp Phe Arg Asp Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg
                570                 575                 580

Glu Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Arg
            585                 590                 595
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Val Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile
        600                 605                 610

Gln Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Cys Glu Lys
    615                 620                 625

Glu Ala Leu Met Ser Glu Leu Lys Met Met Thr His Leu Gly His His
630                 635                 640                 645

Asp Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Val
                650                 655                 660

Tyr Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu
            665                 670                 675

Arg Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys
        680                 685                 690

Glu His Asn Phe Ser Ser Tyr Pro Thr Phe Gln Ala His Ser Asn Ser
    695                 700                 705

Ser Met Pro Gly Ser Arg Glu Val Gln Leu His Pro Pro Leu Asp Gln
710                 715                 720                 725

Leu Ser Gly Phe Asn Gly Asn Ser Ile His Ser Glu Asp Glu Ile Glu
                730                 735                 740

Tyr Glu Asn Gln Lys Arg Leu Ala Glu Glu Glu Glu Glu Asp Leu Asn
            745                 750                 755

Val Leu Thr Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys
        760                 765                 770

Gly Met Glu Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala
    775                 780                 785

Ala Arg Asn Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp
```

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790                 795                 800                 805

Phe Gly Leu Ala Arg Asp Ile Leu Ser Asp Ser Ser Tyr Val Val Arg
                810                 815                 820

Gly Asn Ala Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe
            825                 830                 835

Glu Gly Ile Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu
        840                 845                 850

Leu Trp Glu Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro
    855                 860                 865

Val Asp Ala Asn Phe Tyr Lys Leu Ile Gln Ser Gly Phe Lys Met Glu
870                 875                 880                 885

Gln Pro Phe Tyr Ala Thr Glu Gly Ile Tyr Phe Val Met Gln Ser Cys
                890                 895                 900

Trp Ala Phe Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser
            905                 910                 915

Phe Leu Gly Cys Gln Leu Ala Glu Ala Glu Glu Ala Cys Ile Arg Thr
        920                 925                 930

Ser Ile His Leu Pro Lys Gln Ala Ala Pro Gln Gln Arg Gly Gly Leu
    935                 940                 945

Arg Ala Gln Ser Pro Gln Arg Gln Val Lys Ile His Arg Glu Arg Ser
950                 955                 960                 965
```

(2) INFORMATION FOR SEQ ID NO:3:

```
(i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 3501 base pairs
     (B) TYPE: nucleic acid
     (C) STRANDEDNESS: double
     (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
     (A) NAME/KEY: CDS
     (B) LOCATION: 58..3039

(ix) FEATURE:
     (A) NAME/KEY: mat_peptide
     (B) LOCATION: 139..3036

(ix) FEATURE:
     (A) NAME/KEY: sig_peptide
     (B) LOCATION: 58..138

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGAGGCGGCA TCCGAGGGCT GGGCCGGCGC CCTGGGGGAC CCCGGGCTCC GGAGGCC         57

ATG CCG GCG TTG GCG CGC GAC GCG GGC ACC GTG CCG CTG CTC GTT GTT       105
Met Pro Ala Leu Ala Arg Asp Ala Gly Thr Val Pro Leu Leu Val Val
-27     -25                 -20                 -15

TTT TCT GCA ATG ATA TTT GGG ACT ATT ACA AAT CAA GAT CTG CCT GTG       153
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Phe Ser Ala Met Ile Phe Gly Thr Ile Thr Asn Gln Asp Leu Pro Val
    -10                 -5                   1               5

ATC AAG TGT GTT TTA ATC AAT CAT AAG AAC AAT GAT TCA TCA GTG GGG      201
Ile Lys Cys Val Leu Ile Asn His Lys Asn Asn Asp Ser Ser Val Gly
                10                  15                  20

AAG TCA TCA TCA TAT CCC ATG GTA TCA GAA TCC CCG GAA GAC CTC GGG      249
Lys Ser Ser Ser Tyr Pro Met Val Ser Glu Ser Pro Glu Asp Leu Gly
            25                  30                  35

TGT GCG TTG AGA CCC CAG AGC TCA GGG ACA GTG TAC GAA GCT GCC GCT      297
Cys Ala Leu Arg Pro Gln Ser Ser Gly Thr Val Tyr Glu Ala Ala Ala
        40                  45                  50

GTG GAA GTG GAT GTA TCT GCT TCC ATC ACA CTG CAA GTG CTG GTC GAT      345
Val Glu Val Asp Val Ser Ala Ser Ile Thr Leu Gln Val Leu Val Asp
    55                  60                  65

GCC CCA GGG AAC ATT TCC TGT CTC TGG GTC TTT AAG CAC AGC TCC CTG      393
Ala Pro Gly Asn Ile Ser Cys Leu Trp Val Phe Lys His Ser Ser Leu
70                  75                  80                  85

AAT TGC CAG CCA CAT TTT GAT TTA CAA AAC AGA GGA GTT GTT TCC ATG      441
Asn Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Val Val Ser Met
                90                  95                  100

GTC ATT TTG AAA ATG ACA GAA ACC CAA GCT GGA GAA TAC CTA CTT TTT      489
Val Ile Leu Lys Met Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu Phe
            105                 110                 115

ATT CAG AGT GAA GCT ACC AAT TAC ACA ATA TTG TTT ACA GTG AGT ATA      537
Ile Gln Ser Glu Ala Thr Asn Tyr Thr Ile Leu Phe Thr Val Ser Ile
        120                 125                 130

AGA AAT ACC CTG CTT TAC ACA TTA AGA AGA CCT TAC TTT AGA AAA ATG      585
Arg Asn Thr Leu Leu Tyr Thr Leu Arg Arg Pro Tyr Phe Arg Lys Met
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    135                 140                 145

GAA AAC CAG GAC GCC CTG GTC TGC ATA TCT GAG AGC GTT CCA GAG CCG      633
Glu Asn Gln Asp Ala Leu Val Cys Ile Ser Glu Ser Val Pro Glu Pro
150                 155                 160                 165

ATC GTG GAA TGG GTG CTT TGC GAT TCA CAG GGG GAA AGC TGT AAA GAA      681
Ile Val Glu Trp Val Leu Cys Asp Ser Gln Gly Glu Ser Cys Lys Glu
                170                 175                 180

GAA AGT CCA GCT GTT GTT AAA AAG GAG GAA AAA GTG CTT CAT GAA TTA      729
Glu Ser Pro Ala Val Val Lys Lys Glu Glu Lys Val Leu His Glu Leu
            185                 190                 195

TTT GGG ACG GAC ATA AGG TGC TGT GCC AGA AAT GAA CTG GGC AGG GAA      777
Phe Gly Thr Asp Ile Arg Cys Cys Ala Arg Asn Glu Leu Gly Arg Glu
        200                 205                 210

TGC ACC AGG CTG TTC ACA ATA GAT CTA AAT CAA ACT CCT CAG ACC ACA      825
Cys Thr Arg Leu Phe Thr Ile Asp Leu Asn Gln Thr Pro Gln Thr Thr
    215                 220                 225

TTG CCA CAA TTA TTT CTT AAA GTA GGG GAA CCC TTA TGG ATA AGG TGC      873
Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg Cys
230                 235                 240                 245

AAA GCT GTT CAT GTG AAC CAT GGA TTC GGG CTC ACC TGG GAA TTA GAA      921
Lys Ala Val His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu Glu
                250                 255                 260

AAC AAA GCA CTC GAG GAG GGC AAC TAC TTT GAG ATG AGT ACC TAT TCA      969
Asn Lys Ala Leu Glu Glu Gly Asn Tyr Phe Glu Met Ser Thr Tyr Ser
            265                 270                 275

ACA AAC AGA ACT ATG ATA CGG ATT CTG TTT GCT TTT GTA TCA TCA GTG     1017
Thr Asn Arg Thr Met Ile Arg Ile Leu Phe Ala Phe Val Ser Ser Val
        280                 285                 290
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GCA AGA AAC GAC ACC GGA TAC TAC ACT TGT TCC TCT TCA AAG CAT CCC    1065
Ala Arg Asn Asp Thr Gly Tyr Tyr Thr Cys Ser Ser Ser Lys His Pro
    295                 300                 305

AGT CAA TCA GCT TTG GTT ACC ATC GTA GGA AAG GGA TTT ATA AAT GCT    1113
Ser Gln Ser Ala Leu Val Thr Ile Val Gly Lys Gly Phe Ile Asn Ala
310                 315                 320                 325

ACC AAT TCA AGT GAA GAT TAT GAA ATT GAC CAA TAT GAA GAG TTT TGT    1161
Thr Asn Ser Ser Glu Asp Tyr Glu Ile Asp Gln Tyr Glu Glu Phe Cys
                330                 335                 340

TTT TCT GTC AGG TTT AAA GCC TAC CCA CAA ATC AGA TGT ACG TGG ACC    1209
Phe Ser Val Arg Phe Lys Ala Tyr Pro Gln Ile Arg Cys Thr Trp Thr
            345                 350                 355

TTC TCT CGA AAA TCA TTT CCT TGT GAG CAA AAG GGT CTT GAT AAC GGA    1257
Phe Ser Arg Lys Ser Phe Pro Cys Glu Gln Lys Gly Leu Asp Asn Gly
        360                 365                 370

TAC AGC ATA TCC AAG TTT TGC AAT CAT AAG CAC CAG CCA GGA GAA TAT    1305
Tyr Ser Ile Ser Lys Phe Cys Asn His Lys His Gln Pro Gly Glu Tyr
    375                 380                 385

ATA TTC CAT GCA GAA AAT GAT GAT GCC CAA TTT ACC AAA ATG TTC ACG    1353
Ile Phe His Ala Glu Asn Asp Asp Ala Gln Phe Thr Lys Met Phe Thr
390                 395                 400                 405

CTG AAT ATA AGA AGG AAA CCT CAA GTG CTC GCA GAA GCA TCG GCA AGT    1401
Leu Asn Ile Arg Arg Lys Pro Gln Val Leu Ala Glu Ala Ser Ala Ser
                410                 415                 420

CAG GCG TCC TGT TTC TCG GAT GGA TAC CCA TTA CCA TCT TGG ACC TGG    1449
Gln Ala Ser Cys Phe Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr Trp
            425                 430                 435

AAG AAG TGT TCA GAC AAG TCT CCC AAC TGC ACA GAA GAG ATC ACA GAA    1497
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Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Thr Glu
        440                 445                 450

GGA GTC TGG AAT AGA AAG GCT AAC AGA AAA GTG TTT GGA CAG TGG GTG      1545
Gly Val Trp Asn Arg Lys Ala Asn Arg Lys Val Phe Gly Gln Trp Val
    455                 460                 465

TCG AGC AGT ACT CTA AAC ATG AGT GAA GCC ATA AAA GGG TTC CTG GTC      1593
Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Ile Lys Gly Phe Leu Val
470                 475                 480                 485

AAG TGC TGT GCA TAC AAT TCC CTT GGC ACA TCT TGT GAG ACG ATC CTT      1641
Lys Cys Cys Ala Tyr Asn Ser Leu Gly Thr Ser Cys Glu Thr Ile Leu
                490                 495                 500

TTA AAC TCT CCA GGC CCC TTC CCT TTC ATC CAA GAC AAC ATC TCA TTC      1689
Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser Phe
            505                 510                 515

TAT GCA ACA ATT GGT GTT TGT CTC CTC TTC ATT GTC GTT TTA ACC CTG      1737
Tyr Ala Thr Ile Gly Val Cys Leu Leu Phe Ile Val Val Leu Thr Leu
        520                 525                 530

CTA ATT TGT CAC AAG TAC AAA AAG CAA TTT AGG TAT GAA AGC CAG CTA      1785
Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln Leu
    535                 540                 545

CAG ATG GTA CAG GTG ACC GGC TCC TCA GAT AAT GAG TAC TTC TAC GTT      1833
Gln Met Val Gln Val Thr Gly Ser Ser Asp Asn Glu Tyr Phe Tyr Val
550                 555                 560                 565

GAT TTC AGA GAA TAT GAA TAT GAT CTC AAA TGG GAG TTT CCA AGA GAA      1881
Asp Phe Arg Glu Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg Glu
                570                 575                 580

AAT TTA GAG TTT GGG AAG GTA CTA GGA TCA GGT GCT TTT GGA AAA GTG      1929
Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Lys Val
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                585                     590                     595

ATG AAC GCA ACA GCT TAT GGA ATT AGC AAA ACA GGA GTC TCA ATC CAG      1977
Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile Gln
        600                     605                     610

GTT GCC GTC AAA ATG CTG AAA GAA AAA GCA GAC AGC TCT GAA AGA GAG      2025
Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Ser Glu Arg Glu
    615                     620                     625

GCA CTC ATG TCA GAA CTC AAG ATG ATG ACC CAG CTG GGA AGC CAC GAG      2073
Ala Leu Met Ser Glu Leu Lys Met Met Thr Gln Leu Gly Ser His Glu
630                     635                     640                     645

AAT ATT GTG AAC CTG CTG GGG GCG TGC ACA CTG TCA GGA CCA ATT TAC      2121
Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Ile Tyr
                650                     655                     660

TTG ATT TTT GAA TAC TGT TGC TAT GGT GAT CTT CTC AAC TAT CTA AGA      2169
Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu Arg
            665                     670                     675

AGT AAA AGA GAA AAA TTT CAC AGG ACT TGG ACA GAG ATT TTC AAG GAA      2217
Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys Glu
        680                     685                     690

CAC AAT TTC AGT TTT TAC CCC ACT TTC CAA TCA CAT CCA AAT TCC AGC      2265
His Asn Phe Ser Phe Tyr Pro Thr Phe Gln Ser His Pro Asn Ser Ser
    695                     700                     705

ATG CCT GGT TCA AGA GAA GTT CAG ATA CAC CCG GAC TCG GAT CAA ATC      2313
Met Pro Gly Ser Arg Glu Val Gln Ile His Pro Asp Ser Asp Gln Ile
710                     715                     720                     725

TCA GGG CTT CAT GGG AAT TCA TTT CAC TCT GAA GAT GAA ATT GAA TAT      2361
Ser Gly Leu His Gly Asn Ser Phe His Ser Glu Asp Glu Ile Glu Tyr
                730                     735                     740
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GAA AAC CAA AAA AGG CTG GAA GAA GAG GAG GAC TTG AAT GTG CTT ACA      2409
Glu Asn Gln Lys Arg Leu Glu Glu Glu Glu Asp Leu Asn Val Leu Thr
            745                 750                 755

TTT GAA GAT CTT CTT TGC TTT GCA TAT CAA GTT GCC AAA GGA ATG GAA      2457
Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu
        760                 765                 770

TTT CTG GAA TTT AAG TCG TGT GTT CAC AGA GAC CTG GCC GCC AGG AAC      2505
Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn
    775                 780                 785

GTG CTT GTC ACC CAC GGG AAA GTG GTG AAG ATA TGT GAC TTT GGA TTG      2553
Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu
790                 795                 800                 805

GCT CGA GAT ATC ATG AGT GAT TCC AAC TAT GTT GTC AGG GGC AAT GCC      2601
Ala Arg Asp Ile Met Ser Asp Ser Asn Tyr Val Val Arg Gly Asn Ala
                810                 815                 820

CGT CTG CCT GTA AAA TGG ATG GCC CCC GAA AGC CTG TTT GAA GGC ATC      2649
Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile
            825                 830                 835

TAC ACC ATT AAG AGT GAT GTC TGG TCA TAT GGA ATA TTA CTG TGG GAA      2697
Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu
        840                 845                 850

ATC TTC TCA CTT GGT GTG AAT CCT TAC CCT GGC ATT CCG GTT GAT GCT      2745
Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala
    855                 860                 865

AAC TTC TAC AAA CTG ATT CAA AAT GGA TTT AAA ATG GAT CAG CCA TTT      2793
Asn Phe Tyr Lys Leu Ile Gln Asn Gly Phe Lys Met Asp Gln Pro Phe
870                 875                 880                 885

TAT GCT ACA GAA GAA ATA TAC ATT ATA ATG CAA TCC TGC TGG GCT TTT      2841
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Tyr Ala Thr Glu Glu Ile Tyr Ile Ile Met Gln Ser Cys Trp Ala Phe
                890                 895                 900

GAC TCA AGG AAA CGG CCA TCC TTC CCT AAT TTG ACT TCG TTT TTA GGA      2889
Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly
            905                 910                 915

TGT CAG CTG GCA GAT GCA GAA GAA GCG ATG TAT CAG AAT GTG GAT GGC      2937
Cys Gln Leu Ala Asp Ala Glu Glu Ala Met Tyr Gln Asn Val Asp Gly
        920                 925                 930

CGT GTT TCG GAA TGT CCT CAC ACC TAC CAA AAC AGG CGA CCT TTC AGC      2985
Arg Val Ser Glu Cys Pro His Thr Tyr Gln Asn Arg Arg Pro Phe Ser
    935                 940                 945

AGA GAG ATG GAT TTG GGG CTA CTC TCT CCG CAG GCT CAG GTC GAA GAT      3033
Arg Glu Met Asp Leu Gly Leu Leu Ser Pro Gln Ala Gln Val Glu Asp
950                 955                 960                 965

TCG TAGAGGAACA ATTTAGTTTT AAGGACTTCA TCCCTCCACC TATCCCTAAC           3086
Ser

AGGCTGTAGA TTACCAAAAC AAGATTAATT TCATCACTAA AAGAAAATCT ATTATCAACT    3146

GCTGCTTCAC CAGACTTTTC TCTAGAAGCC GTCTGCGTTT ACTCTTGTTT TCAAAGGGAC    3206

TTTTGTAAAA TCAAATCATC CTGTCACAAG GCAGGAGGAG CTGATAATGA ACTTTATTGG    3266

AGCATTGATC TGCATCCAAG GCCTTCTCAG GCCGGCTTGA GTGAATTGTG TACCTGAAGT    3326

ACAGTATATT CTTGTAAATA CATAAAACAA AAGCATTTTG CTAAGGAGAA GCTAATATGA    3386

TTTTTTAAGT CTATGTTTTA AAATAATATG TAAATTTTTC AGCTATTTAG TGATATATTT    3446

TATGGGTGGG AATAAAATTT CTACTACAGA AAAAAAAAAA AAAAAAAAAA AAAAA         3501
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 993 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Pro Ala Leu Ala Arg Asp Ala Gly Thr Val Pro Leu Leu Val Val
-27     -25                 -20                 -15

Phe Ser Ala Met Ile Phe Gly Thr Ile Thr Asn Gln Asp Leu Pro Val
    -10                  -5                   1                   5

Ile Lys Cys Val Leu Ile Asn His Lys Asn Asn Asp Ser Ser Val Gly
                 10                  15                  20

Lys Ser Ser Ser Tyr Pro Met Val Ser Glu Ser Pro Glu Asp Leu Gly
             25                  30                  35

Cys Ala Leu Arg Pro Gln Ser Ser Gly Thr Val Tyr Glu Ala Ala Ala
         40                  45                  50

Val Glu Val Asp Val Ser Ala Ser Ile Thr Leu Gln Val Leu Val Asp
     55                  60                  65

Ala Pro Gly Asn Ile Ser Cys Leu Trp Val Phe Lys His Ser Ser Leu
 70                  75                  80                  85

Asn Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Val Val Ser Met
                 90                  95                 100

Val Ile Leu Lys Met Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu Phe
            105                 110                 115
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Ile Gln Ser Glu Ala Thr Asn Tyr Thr Ile Leu Phe Thr Val Ser Ile
        120                 125                 130

Arg Asn Thr Leu Leu Tyr Thr Leu Arg Arg Pro Tyr Phe Arg Lys Met
    135                 140                 145

Glu Asn Gln Asp Ala Leu Val Cys Ile Ser Glu Ser Val Pro Glu Pro
150                 155                 160                 165

Ile Val Glu Trp Val Leu Cys Asp Ser Gln Gly Glu Ser Cys Lys Glu
                170                 175                 180

Glu Ser Pro Ala Val Val Lys Lys Glu Glu Lys Val Leu His Glu Leu
            185                 190                 195

Phe Gly Thr Asp Ile Arg Cys Cys Ala Arg Asn Glu Leu Gly Arg Glu
        200                 205                 210

Cys Thr Arg Leu Phe Thr Ile Asp Leu Asn Gln Thr Pro Gln Thr Thr
    215                 220                 225

Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg Cys
230                 235                 240                 245

Lys Ala Val His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu Glu
                250                 255                 260

Asn Lys Ala Leu Glu Glu Gly Asn Tyr Phe Glu Met Ser Thr Tyr Ser
            265                 270                 275

Thr Asn Arg Thr Met Ile Arg Ile Leu Phe Ala Phe Val Ser Ser Val
        280                 285                 290

Ala Arg Asn Asp Thr Gly Tyr Tyr Thr Cys Ser Ser Ser Lys His Pro
    295                 300                 305

Ser Gln Ser Ala Leu Val Thr Ile Val Gly Lys Gly Phe Ile Asn Ala
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310                 315                 320                 325

Thr Asn Ser Ser Glu Asp Tyr Glu Ile Asp Gln Tyr Glu Glu Phe Cys
                330                 335                 340

Phe Ser Val Arg Phe Lys Ala Tyr Pro Gln Ile Arg Cys Thr Trp Thr
            345                 350                 355

Phe Ser Arg Lys Ser Phe Pro Cys Glu Gln Lys Gly Leu Asp Asn Gly
            360                 365                 370

Tyr Ser Ile Ser Lys Phe Cys Asn His Lys His Gln Pro Gly Glu Tyr
    375                 380                 385

Ile Phe His Ala Glu Asn Asp Asp Ala Gln Phe Thr Lys Met Phe Thr
390                 395                 400                 405

Leu Asn Ile Arg Arg Lys Pro Gln Val Leu Ala Glu Ala Ser Ala Ser
                410                 415                 420

Gln Ala Ser Cys Phe Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr Trp
            425                 430                 435

Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Thr Glu
        440                 445                 450

Gly Val Trp Asn Arg Lys Ala Asn Arg Lys Val Phe Gly Gln Trp Val
    455                 460                 465

Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Ile Lys Gly Phe Leu Val
470                 475                 480                 485

Lys Cys Cys Ala Tyr Asn Ser Leu Gly Thr Ser Cys Glu Thr Ile Leu
                490                 495                 500

Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser Phe
            505                 510                 515
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Tyr Ala Thr Ile Gly Val Cys Leu Leu Phe Ile Val Val Leu Thr Leu
        520                 525                 530

Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln Leu
    535                 540                 545

Gln Met Val Gln Val Thr Gly Ser Ser Asp Asn Glu Tyr Phe Tyr Val
550                 555                 560                 565

Asp Phe Arg Glu Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg Glu
                570                 575                 580

Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Lys Val
            585                 590                 595

Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile Gln
        600                 605                 610

Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Ser Glu Arg Glu
    615                 620                 625

Ala Leu Met Ser Glu Leu Lys Met Met Thr Gln Leu Gly Ser His Glu
630                 635                 640                 645

Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Ile Tyr
                650                 655                 660

Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu Arg
            665                 670                 675

Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys Glu
        680                 685                 690

His Asn Phe Ser Phe Tyr Pro Thr Phe Gln Ser His Pro Asn Ser Ser
    695                 700                 705

Met Pro Gly Ser Arg Glu Val Gln Ile His Pro Asp Ser Asp Gln Ile
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710                     715                     720                     725
Ser Gly Leu His Gly Asn Ser Phe His Ser Glu Asp Glu Ile Glu Tyr
                730                     735                     740
Glu Asn Gln Lys Arg Leu Glu Glu Glu Glu Asp Leu Asn Val Leu Thr
            745                     750                     755
Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu
        760                     765                     770
Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn
    775                     780                     785
Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu
790                     795                     800                     805
Ala Arg Asp Ile Met Ser Asp Ser Asn Tyr Val Val Arg Gly Asn Ala
                810                     815                     820
Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile
            825                     830                     835
Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu
        840                     845                     850
Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala
    855                     860                     865
Asn Phe Tyr Lys Leu Ile Gln Asn Gly Phe Lys Met Asp Gln Pro Phe
870                     875                     880                     885
Tyr Ala Thr Glu Glu Ile Tyr Ile Ile Met Gln Ser Cys Trp Ala Phe
                890                     895                     900
Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly
            905                     910                     915
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Cys Gln Leu Ala Asp Ala Glu Glu Ala Met Tyr Gln Asn Val Asp Gly
        920                 925                 930

Arg Val Ser Glu Cys Pro His Thr Tyr Gln Asn Arg Arg Pro Phe Ser
    935                 940                 945

Arg Glu Met Asp Leu Gly Leu Leu Ser Pro Gln Ala Gln Val Glu Asp
950                 955                 960                 965

Ser
 (2) INFORMATION FOR SEQ ID NO:5:

      (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 5406 base pairs
           (B) TYPE: nucleic acid
           (C) STRANDEDNESS: double
           (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: cDNA

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

      (v) FRAGMENT TYPE: N-terminal

     (ix) FEATURE:
           (A) NAME/KEY: CDS
           (B) LOCATION: 208..4311

     (ix) FEATURE:
           (A) NAME/KEY: mat_peptide
           (B) LOCATION: 265..4308

     (ix) FEATURE:
           (A) NAME/KEY: sig_peptide
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(B) LOCATION: 208..264

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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CTGTGTCCCG CAGCCGGATA ACCTGGCTGA CCCGATTCCG CGGACACCCG TGCAGCCGCG      60

GCTGGAGCCA GGGCGCCGGT GCCCGCGCTC TCCCCGGTCT TGCGCTGCGG GGGCCGATAC     120

CGCCTCTGTG ACTTCTTTGC GGGCCAGGGA CGGAGAAGGA GTCTGTGCCT GAGAAACTGG     180

GCTCTGTGCC CAGGCGCGAG GTGCAGG ATG GAG AGC AAG GGC CTG CTA GCT         231
                              Met Glu Ser Lys Gly Leu Leu Ala
                              -19             -15

GTC GCT CTG TGG TTC TGC GTG GAG ACC CGA GCC GCC TCT GTG GGT TTG       279
Val Ala Leu Trp Phe Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu
    -10                 -5                  1                   5

CCT GGC GAT TTT CTC CAT CCC CCC AAG CTC AGC ACA CAG AAA GAC ATA       327
Pro Gly Asp Phe Leu His Pro Pro Lys Leu Ser Thr Gln Lys Asp Ile
                10                  15                  20

CTG ACA ATT TTG GCA AAT ACA ACC CTT CAG ATT ACT TGC AGG GGA CAG       375
Leu Thr Ile Leu Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln
            25                  30                  35

CGG GAC CTG GAC TGG CTT TGG CCC AAT GCT CAG CGT GAT TCT GAG GAA       423
Arg Asp Leu Asp Trp Leu Trp Pro Asn Ala Gln Arg Asp Ser Glu Glu
        40                  45                  50

AGG GTA TTG GTG ACT GAA TGC GGC GGT GGT GAC AGT ATC TTC TGC AAA       471
Arg Val Leu Val Thr Glu Cys Gly Gly Gly Asp Ser Ile Phe Cys Lys
    55                  60                  65

ACA CTC ACC ATT CCC AGG GTG GTT GGA AAT GAT ACT GGA GCC TAC AAG       519
Thr Leu Thr Ile Pro Arg Val Val Gly Asn Asp Thr Gly Ala Tyr Lys
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85

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 70                  75                  80                  85

TGC TCG TAC CGG GAC GTC GAC ATA GCC TCC ACT GTT TAT GTC TAT GTT      567
Cys Ser Tyr Arg Asp Val Asp Ile Ala Ser Thr Val Tyr Val Tyr Val
                 90                  95                 100

CGA GAT TAC AGA TCA CCA TTC ATC GCC TCT GTC AGT GAC CAG CAT GGC      615
Arg Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp Gln His Gly
            105                 110                 115

ATC GTG TAC ATC ACC GAG AAC AAG AAC AAA ACT GTG GTG ATC CCC TGC      663
Ile Val Tyr Ile Thr Glu Asn Lys Asn Lys Thr Val Val Ile Pro Cys
        120                 125                 130

CGA GGG TCG ATT TCA AAC CTC AAT GTG TCT CTT TGC GCT AGG TAT CCA      711
Arg Gly Ser Ile Ser Asn Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro
    135                 140                 145

GAA AAG AGA TTT GTT CCG GAT GGA AAC AGA ATT TCC TGG GAC AGC GAG      759
Glu Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp Asp Ser Glu
150                 155                 160                 165

ATA GGC TTT ACT CTC CCC AGT TAC ATG ATC AGC TAT GCC GGC ATG GTC      807
Ile Gly Phe Thr Leu Pro Ser Tyr Met Ile Ser Tyr Ala Gly Met Val
                170                 175                 180

TTC TGT GAG GCA AAG ATC AAT GAT GAA ACC TAT CAG TCT ATC ATG TAC      855
Phe Cys Glu Ala Lys Ile Asn Asp Glu Thr Tyr Gln Ser Ile Met Tyr
            185                 190                 195

ATA GTT GTG GTT GTA GGA TAT AGG ATT TAT GAT GTG ATT CTG AGC CCC      903
Ile Val Val Val Val Gly Tyr Arg Ile Tyr Asp Val Ile Leu Ser Pro
        200                 205                 210

CCG CAT GAA ATT GAG CTA TCT GCC GGA GAA AAA CTT GTC TTA AAT TGT      951
Pro His Glu Ile Glu Leu Ser Ala Gly Glu Lys Leu Val Leu Asn Cys
    215                 220                 225
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ACA GCG AGA ACA GAG CTC AAT GTG GGG CTT GAT TTC ACC TGG CAC TCT      999
Thr Ala Arg Thr Glu Leu Asn Val Gly Leu Asp Phe Thr Trp His Ser
230                 235                 240                 245

CCA CCT TCA AAG TCT CAT CAT AAG AAG ATT GTA AAC CGG GAT GTG AAA     1047
Pro Pro Ser Lys Ser His His Lys Lys Ile Val Asn Arg Asp Val Lys
                250                 255                 260

CCC TTT CCT GGG ACT GTG GCG AAG ATG TTT TTG AGC ACC TTG ACA ATA     1095
Pro Phe Pro Gly Thr Val Ala Lys Met Phe Leu Ser Thr Leu Thr Ile
            265                 270                 275

GAA AGT GTG ACC AAG AGT GAC CAA GGG GAA TAC ACC TGT GTA GCG TCC     1143
Glu Ser Val Thr Lys Ser Asp Gln Gly Glu Tyr Thr Cys Val Ala Ser
        280                 285                 290

AGT GGA CGG ATG ATC AAG AGA AAT AGA ACA TTT GTC CGA GTT CAC ACA     1191
Ser Gly Arg Met Ile Lys Arg Asn Arg Thr Phe Val Arg Val His Thr
    295                 300                 305

AAG CCT TTT ATT GCT TTC GGT AGT GGG ATG AAA TCT TTG GTG GAA GCC     1239
Lys Pro Phe Ile Ala Phe Gly Ser Gly Met Lys Ser Leu Val Glu Ala
310                 315                 320                 325

ACA GTG GGC AGT CAA GTC CGA ATC CCT GTG AAG TAT CTC AGT TAC CCA     1287
Thr Val Gly Ser Gln Val Arg Ile Pro Val Lys Tyr Leu Ser Tyr Pro
                330                 335                 340

GCT CCT GAT ATC AAA TGG TAC AGA AAT GGA AGG CCC ATT GAG TCC AAC     1335
Ala Pro Asp Ile Lys Trp Tyr Arg Asn Gly Arg Pro Ile Glu Ser Asn
            345                 350                 355

TAC ACA ATG ATT GTT GGC GAT GAA CTC ACC ATC ATG GAA GTG ACT GAA     1383
Tyr Thr Met Ile Val Gly Asp Glu Leu Thr Ile Met Glu Val Thr Glu
        360                 365                 370

AGA GAT GCA GGA AAC TAC ACG GTC ATC CTC ACC AAC CCC ATT TCA ATG     1431
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Arg Asp Ala Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Met
    375                 380                 385

GAG AAA CAG AGC CAC ATG GTC TCT CTG GTT GTG AAT GTC CCA CCC CAG      1479
Glu Lys Gln Ser His Met Val Ser Leu Val Val Asn Val Pro Pro Gln
390                 395                 400                 405

ATC GGT GAG AAA GCC TTG ATC TCG CCT ATG GAT TCC TAC CAG TAT GGG      1527
Ile Gly Glu Lys Ala Leu Ile Ser Pro Met Asp Ser Tyr Gln Tyr Gly
                410                 415                 420

ACC ATG CAG ACA TTG ACA TGC ACA GTC TAC GCC AAC CCT CCC CTG CAC      1575
Thr Met Gln Thr Leu Thr Cys Thr Val Tyr Ala Asn Pro Pro Leu His
            425                 430                 435

CAC ATC CAG TGG TAC TGG CAG CTA GAA GAA GCC TGC TCC TAC AGA CCC      1623
His Ile Gln Trp Tyr Trp Gln Leu Glu Glu Ala Cys Ser Tyr Arg Pro
        440                 445                 450

GGC CAA ACA AGC CCG TAT GCT TGT AAA GAA TGG AGA CAC GTG GAG GAT      1671
Gly Gln Thr Ser Pro Tyr Ala Cys Lys Glu Trp Arg His Val Glu Asp
    455                 460                 465

TTC CAG GGG GGA AAC AAG ATC GAA GTC ACC AAA AAC CAA TAT GCC CTG      1719
Phe Gln Gly Gly Asn Lys Ile Glu Val Thr Lys Asn Gln Tyr Ala Leu
470                 475                 480                 485

ATT GAA GGA AAA AAC AAA ACT GTA AGT ACG CTG GTC ATC CAA GCT GCC      1767
Ile Glu Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala
                490                 495                 500

AAC GTG TCA GCG TTG TAC AAA TGT GAA GCC ATC AAC AAA GCG GGA CGA      1815
Asn Val Ser Ala Leu Tyr Lys Cys Glu Ala Ile Asn Lys Ala Gly Arg
            505                 510                 515

GGA GAG AGG GTC ATC TCC TTC CAT GTG ATC AGG GGT CCT GAA ATT ACT      1863
Gly Glu Arg Val Ile Ser Phe His Val Ile Arg Gly Pro Glu Ile Thr
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        520                     525                     530

GTG CAA CCT GCT GCC CAG CCA ACT GAG CAG GAG AGT GTG TCC CTG TTG        1911
Val Gln Pro Ala Ala Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Leu
    535                 540                     545

TGC ACT GCA GAC AGA AAT ACG TTT GAG AAC CTC ACG TGG TAC AAG CTT        1959
Cys Thr Ala Asp Arg Asn Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu
550                     555                 560                 565

GGC TCA CAG GCA ACA TCG GTC CAC ATG GGC GAA TCA CTC ACA CCA GTT        2007
Gly Ser Gln Ala Thr Ser Val His Met Gly Glu Ser Leu Thr Pro Val
                570                     575                 580

TGC AAG AAC TTG GAT GCT CTT TGG AAA CTG AAT GGC ACC ATG TTT TCT        2055
Cys Lys Asn Leu Asp Ala Leu Trp Lys Leu Asn Gly Thr Met Phe Ser
            585                 590                     595

AAC AGC ACA AAT GAC ATC TTG ATT GTG GCA TTT CAG AAT GCC TCT CTG        2103
Asn Ser Thr Asn Asp Ile Leu Ile Val Ala Phe Gln Asn Ala Ser Leu
        600                     605                     610

CAG GAC CAA GGC GAC TAT GTT TGC TCT GCT CAA GAT AAG AAG ACC AAG        2151
Gln Asp Gln Gly Asp Tyr Val Cys Ser Ala Gln Asp Lys Lys Thr Lys
    615                 620                     625

AAA AGA CAT TGC CTG GTC AAA CAG CTC ATC ATC CTA GAG CGC ATG GCA        2199
Lys Arg His Cys Leu Val Lys Gln Leu Ile Ile Leu Glu Arg Met Ala
630                     635                 640                     645

CCC ATG ATC ACC GGA AAT CTG GAG AAT CAG ACA ACA ACC ATT GGC GAG        2247
Pro Met Ile Thr Gly Asn Leu Glu Asn Gln Thr Thr Thr Ile Gly Glu
                650                     655                 660

ACC ATT GAA GTG ACT TGC CCA GCA TCT GGA AAT CCT ACC CCA CAC ATT        2295
Thr Ile Glu Val Thr Cys Pro Ala Ser Gly Asn Pro Thr Pro His Ile
            665                 670                     675
```

```
ACA TGG TTC AAA GAC AAC GAG ACC CTG GTA GAA GAT TCA GGC ATT GTA      2343
Thr Trp Phe Lys Asp Asn Glu Thr Leu Val Glu Asp Ser Gly Ile Val
        680                 685                 690

CTG AGA GAT GGG AAC CGG AAC CTG ACT ATC CGC AGG GTG AGG AAG GAG      2391
Leu Arg Asp Gly Asn Arg Asn Leu Thr Ile Arg Arg Val Arg Lys Glu
    695                 700                 705

GAT GGA GGC CTC TAC ACC TGC CAG GCC TGC AAT GTC CTT GGC TGT GCA      2439
Asp Gly Gly Leu Tyr Thr Cys Gln Ala Cys Asn Val Leu Gly Cys Ala
710                 715                 720                 725

AGA GCG GAG ACG CTC TTC ATA ATA GAA GGT GCC CAG GAA AAG ACC AAC      2487
Arg Ala Glu Thr Leu Phe Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn
                730                 735                 740

TTG GAA GTC ATT ATC CTC GTC GGC ACT GCA GTG ATT GCC ATG TTC TTC      2535
Leu Glu Val Ile Ile Leu Val Gly Thr Ala Val Ile Ala Met Phe Phe
            745                 750                 755

TGG CTC CTT CTT GTC ATT CTC GTA CGG ACC GTT AAG CGG GCC AAT GAA      2583
Trp Leu Leu Leu Val Ile Leu Val Arg Thr Val Lys Arg Ala Asn Glu
        760                 765                 770

GGG GAA CTG AAG ACA GGC TAC TTG TCT ATT GTC ATG GAT CCA GAT GAA      2631
Gly Glu Leu Lys Thr Gly Tyr Leu Ser Ile Val Met Asp Pro Asp Glu
    775                 780                 785

TTG CCC TTG GAT GAG CGC TGT GAA CGC TTG CCT TAT GAT GCC AGC AAG      2679
Leu Pro Leu Asp Glu Arg Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys
790                 795                 800                 805

TGG GAA TTC CCC AGG GAC CGG CTG AAA CTA GGA AAA CCT CTT GGC CGC      2727
Trp Glu Phe Pro Arg Asp Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg
                810                 815                 820

GGT GCC TTC GGC CAA GTG ATT GAG GCA GAC GCT TTT GGA ATT GAC AAG      2775
```

```
Gly Ala Phe Gly Gln Val Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys
            825                 830                 835

ACA GCG ACT TGC AAA ACA GTA GCC GTC AAG ATG TTG AAA GAA GGA GCA      2823
Thr Ala Thr Cys Lys Thr Val Ala Val Lys Met Leu Lys Glu Gly Ala
        840                 845                 850

ACA CAC AGC GAG CAT CGA GCC CTC ATG TCT GAA CTC AAG ATC CTC ATC      2871
Thr His Ser Glu His Arg Ala Leu Met Ser Glu Leu Lys Ile Leu Ile
    855                 860                 865

CAC ATT GGT CAC CAT CTC AAT GTG GTG AAC CTC CTA GGC GCC TGC ACC      2919
His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly Ala Cys Thr
870                 875                 880                 885

AAG CCG GGA GGG CCT CTC ATG GTG ATT GTG GAA TTC TCG AAG TTT GGA      2967
Lys Pro Gly Gly Pro Leu Met Val Ile Val Glu Phe Ser Lys Phe Gly
                890                 895                 900

AAC CTA TCA ACT TAC TTA CGG GGC AAG AGA AAT GAA TTT GTT CCC TAT      3015
Asn Leu Ser Thr Tyr Leu Arg Gly Lys Arg Asn Glu Phe Val Pro Tyr
            905                 910                 915

AAG AGC AAA GGG GCA CGC TTC CGC CAG GGC AAG GAC TAC GTT GGG GAG      3063
Lys Ser Lys Gly Ala Arg Phe Arg Gln Gly Lys Asp Tyr Val Gly Glu
        920                 925                 930

CTC TCC GTG GAT CTG AAA AGA CGC TTG GAC AGC ATC ACC AGC AGC CAG      3111
Leu Ser Val Asp Leu Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln
    935                 940                 945

AGC TCT GCC AGC TCA GGC TTT GTT GAG GAG AAA TCG CTC AGT GAT GTA      3159
Ser Ser Ala Ser Ser Gly Phe Val Glu Glu Lys Ser Leu Ser Asp Val
950                 955                 960                 965

GAG GAA GAA GAA GCT TCT GAA GAA CTG TAC AAG GAC TTC CTG ACC TTG      3207
Glu Glu Glu Glu Ala Ser Glu Glu Leu Tyr Lys Asp Phe Leu Thr Leu
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                        970                 975                 980

GAG CAT CTC ATC TGT TAC AGC TTC CAA GTG GCT AAG GGC ATG GAG TTC      3255
Glu His Leu Ile Cys Tyr Ser Phe Gln Val Ala Lys Gly Met Glu Phe
            985                 990                 995

TTG GCA TCA AGG AAG TGT ATC CAC AGG GAC CTG GCA GCA CGA AAC ATT      3303
Leu Ala Ser Arg Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile
        1000                1005                1010

CTC CTA TCG GAG AAG AAT GTG GTT AAG ATC TGT GAC TTC GGC TTG GCC      3351
Leu Leu Ser Glu Lys Asn Val Val Lys Ile Cys Asp Phe Gly Leu Ala
    1015                1020                1025

CGG GAC ATT TAT AAA GAC CCG GAT TAT GTC AGA AAA GGA GAT GCC CGA      3399
Arg Asp Ile Tyr Lys Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg
1030                1035                1040                1045

CTC CCT TTG AAG TGG ATG GCC CCG GAA ACC ATT TTT GAC AGA GTA TAC      3447
Leu Pro Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp Arg Val Tyr
                1050                1055                1060

ACA ATT CAG AGC GAT GTG TGG TCT TTC GGT GTG TTG CTC TGG GAA ATA      3495
Thr Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile
            1065                1070                1075

TTT TCC TTA GGT GCC TCC CCA TAC CCT GGG GTC AAG ATT GAT GAA GAA      3543
Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu
        1080                1085                1090

TTT TGT AGG AGA TTG AAA GAA GGA ACT AGA ATG CGG GCT CCT GAC TAC      3591
Phe Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro Asp Tyr
    1095                1100                1105

ACT ACC CCA GAA ATG TAC CAG ACC ATG CTG GAC TGC TGG CAT GAG GAC      3639
Thr Thr Pro Glu Met Tyr Gln Thr Met Leu Asp Cys Trp His Glu Asp
1110                1115                1120                1125
```

```
CCC AAC CAG AGA CCC TCG TTT TCA GAG TTG GTG GAG CAT TTG GGA AAC      3687
Pro Asn Gln Arg Pro Ser Phe Ser Glu Leu Val Glu His Leu Gly Asn
                1130                    1135                1140

CTC CTG CAA GCA AAT GCG CAG CAG GAT GGC AAA GAC TAT ATT GTT CTT      3735
Leu Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys Asp Tyr Ile Val Leu
            1145                    1150                1155

CCA ATG TCA GAG ACA CTG AGC ATG GAA GAG GAT TCT GGA CTC TCC CTG      3783
Pro Met Ser Glu Thr Leu Ser Met Glu Glu Asp Ser Gly Leu Ser Leu
        1160                    1165                1170

CCT ACC TCA CCT GTT TCC TGT ATG GAG GAA GAG GAA GTG TGC GAC CCC      3831
Pro Thr Ser Pro Val Ser Cys Met Glu Glu Glu Glu Val Cys Asp Pro
    1175                    1180                1185

AAA TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAT TAT CTC CAG AAC      3879
Lys Phe His Tyr Asp Asn Thr Ala Gly Ile Ser His Tyr Leu Gln Asn
1190                    1195                1200                1205

AGT AAG CGA AAG AGC CGG CCA GTG AGT GTA AAA ACA TTT GAA GAT ATC      3927
Ser Lys Arg Lys Ser Arg Pro Val Ser Val Lys Thr Phe Glu Asp Ile
                1210                    1215                1220

CCA TTG GAG GAA CCA GAA GTA AAA GTG ATC CCA GAT GAC AGC CAG ACA      3975
Pro Leu Glu Glu Pro Glu Val Lys Val Ile Pro Asp Asp Ser Gln Thr
            1225                    1230                1235

GAC AGT GGG ATG GTC CTT GCA TCA GAA GAG CTG AAA ACT CTG GAA GAC      4023
Asp Ser Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp
        1240                    1245                1250

AGG AAC AAA TTA TCT CCA TCT TTT GGT GGA ATG ATG CCC AGT AAA AGC      4071
Arg Asn Lys Leu Ser Pro Ser Phe Gly Gly Met Met Pro Ser Lys Ser
    1255                    1260                1265

AGG GAG TCT GTG GCC TCG GAA GGC TCC AAC CAG ACC AGT GGC TAC CAG      4119
```

```
Arg Glu Ser Val Ala Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln
1270                1275                1280                1285

TCT GGG TAT CAC TCA GAT GAC ACA GAC ACC ACC GTG TAC TCC AGC GAC      4167
Ser Gly Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr Ser Ser Asp
                1290                1295                1300

GAG GCA GGA CTT TTA AAG ATG GTG GAT GCT GCA GTT CAC GCT GAC TCA      4215
Glu Ala Gly Leu Leu Lys Met Val Asp Ala Ala Val His Ala Asp Ser
            1305                1310                1315

GGG ACC ACA CTG CAG CTC ACC TCC TGT TTA AAT GGA AGT GGT CCT GTC      4263
Gly Thr Thr Leu Gln Leu Thr Ser Cys Leu Asn Gly Ser Gly Pro Val
        1320                1325                1330

CCG GCT CCG CCC CCA ACT CCT GGA AAT CAC GAG AGA GGT GCT GCT TAGATTTTCA   4318
Pro Ala Pro Pro Pro Thr Pro Gly Asn His Glu Arg Gly Ala Ala
    1335                1340                1345

AGTGTTGTTC TTTCCACCAC CCGGAAGTAG CCACATTTGA TTTTCATTTT TGGAGGAGGG    4378

ACCTCAGACT GCAAGGAGCT TGTCCTCAGG GCATTTCCAG AGAAGATGCC CATGACCCAA    4438

GAATGTGTTG ACTCTACTCT CTTTTCCATT CATTTAAAAG TCCTATATAA TGTGCCCTGC    4498

TGTGGTCTCA CTACCAGTTA AAGCAAAAGA CTTTCAAACA CGTGGACTCT GTCCTCCAAG    4558

AAGTGGCAAC GGCACCTCTG TGAAACTGGA TCGAATGGGC AATGCTTTGT GTGTTGAGGA    4618

TGGGTGAGAT GTCCCAGGGC CGAGTCTGTC TACCTTGGAG GCTTTGTGGA GGATGCGGCT    4678

ATGAGCCAAG TGTTAAGTGT GGGATGTGGA CTGGGAGGAA GGAAGGCGCA AGCCGTCCGG    4738

AGAGCGGTTG GAGCCTGCAG ATGCATTGTG CTGGCTCTGG TGGAGGTGGG CTTGTGGCCT    4798

GTCAGGAAAC GCAAAGGCGG CCGGCAGGGT TTGGTTTTGG AAGGTTTGCG TGCTCTTCAC    4858
```

```
AGTCGGGTTA CAGGCGAGTT CCCTGTGGCG TTTCCTACTC CTAATGAGAG TTCCTTCCGG    4918

ACTCTTACGT GTCTCCTGGC CTGGCCCCAG GAAGGAAATG ATGCAGCTTG CTCCTTCCTC    4978

ATCTCTCAGG CTGTGCCTTA ATTCAGAACA CCAAAAGAGA GGAACGTCGG CAGAGGCTCC    5038

TGACGGGGCC GAAGAATTGT GAGAACAGAA CAGAAACTCA GGGTTTCTGC TGGGTGGAGA    5098

CCCACGTGGC GCCCTGGTGG CAGGTCTGAG GGTTCTCTGT CAAGTGGCGG TAAAGGCTCA    5158

GGCTGGTGTT CTTCCTCTAT CTCCACTCCT GTCAGGCCCC CAAGTCCTCA GTATTTTAGC    5218

TTTGTGGCTT CCTGATGGCA GAAAAATCTT AATTGGTTGG TTTGCTCTCC AGATAATCAC    5278

TAGCCAGATT TCGAAATTAC TTTTTAGCCG AGGTTATGAT AACATCTACT GTATCCTTTA    5338

GAATTTTAAC CTATAAAACT ATGTCTACTG GTTTCTGCCT GTGTGCTTAT GTTAAAAAAA    5398

AAAAAAAA                                                              5406
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1367 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Glu Ser Lys Gly Leu Leu Ala Val Ala Leu Trp Phe Cys Val Glu
-19             -15                 -10                  -5

Thr Arg Ala Ala Ser Val Gly Leu Pro Gly Asp Phe Leu His Pro Pro
              1                   5                  10
```

```
Lys Leu Ser Thr Gln Lys Asp Ile Leu Thr Ile Leu Ala Asn Thr Thr
     15                  20                  25

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
 30                  35                  40                  45

Asn Ala Gln Arg Asp Ser Glu Glu Arg Val Leu Val Thr Glu Cys Gly
                 50                  55                  60

Gly Gly Asp Ser Ile Phe Cys Lys Thr Leu Thr Ile Pro Arg Val Val
             65                  70                  75

Gly Asn Asp Thr Gly Ala Tyr Lys Cys Ser Tyr Arg Asp Val Asp Ile
         80                  85                  90

Ala Ser Thr Val Tyr Val Tyr Val Arg Asp Tyr Arg Ser Pro Phe Ile
     95                 100                 105

Ala Ser Val Ser Asp Gln His Gly Ile Val Tyr Ile Thr Glu Asn Lys
110                 115                 120                 125

Asn Lys Thr Val Val Ile Pro Cys Arg Gly Ser Ile Ser Asn Leu Asn
                130                 135                 140

Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly
            145                 150                 155

Asn Arg Ile Ser Trp Asp Ser Glu Ile Gly Phe Thr Leu Pro Ser Tyr
        160                 165                 170

Met Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp
    175                 180                 185

Glu Thr Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg
190                 195                 200                 205

Ile Tyr Asp Val Ile Leu Ser Pro Pro His Glu Ile Glu Leu Ser Ala
```

```
                        210                 215                 220

Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val
            225                 230                 235

Gly Leu Asp Phe Thr Trp His Ser Pro Pro Ser Lys Ser His His Lys
        240                 245                 250

Lys Ile Val Asn Arg Asp Val Lys Pro Phe Pro Gly Thr Val Ala Lys
    255                 260                 265

Met Phe Leu Ser Thr Leu Thr Ile Glu Ser Val Thr Lys Ser Asp Gln
270                 275                 280                 285

Gly Glu Tyr Thr Cys Val Ala Ser Ser Gly Arg Met Ile Lys Arg Asn
                290                 295                 300

Arg Thr Phe Val Arg Val His Thr Lys Pro Phe Ile Ala Phe Gly Ser
            305                 310                 315

Gly Met Lys Ser Leu Val Glu Ala Thr Val Gly Ser Gln Val Arg Ile
        320                 325                 330

Pro Val Lys Tyr Leu Ser Tyr Pro Ala Pro Asp Ile Lys Trp Tyr Arg
    335                 340                 345

Asn Gly Arg Pro Ile Glu Ser Asn Tyr Thr Met Ile Val Gly Asp Glu
350                 355                 360                 365

Leu Thr Ile Met Glu Val Thr Glu Arg Asp Ala Gly Asn Tyr Thr Val
                370                 375                 380

Ile Leu Thr Asn Pro Ile Ser Met Glu Lys Gln Ser His Met Val Ser
            385                 390                 395

Leu Val Val Asn Val Pro Pro Gln Ile Gly Glu Lys Ala Leu Ile Ser
        400                 405                 410
```

```
Pro Met Asp Ser Tyr Gln Tyr Gly Thr Met Gln Thr Leu Thr Cys Thr
    415                 420                 425

Val Tyr Ala Asn Pro Pro Leu His His Ile Gln Trp Tyr Trp Gln Leu
430                 435                 440                 445

Glu Glu Ala Cys Ser Tyr Arg Pro Gly Gln Thr Ser Pro Tyr Ala Cys
                450                 455                 460

Lys Glu Trp Arg His Val Glu Asp Phe Gln Gly Gly Asn Lys Ile Glu
            465                 470                 475

Val Thr Lys Asn Gln Tyr Ala Leu Ile Glu Gly Lys Asn Lys Thr Val
        480                 485                 490

Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr Lys Cys
    495                 500                 505

Glu Ala Ile Asn Lys Ala Gly Arg Gly Glu Arg Val Ile Ser Phe His
510                 515                 520                 525

Val Ile Arg Gly Pro Glu Ile Thr Val Gln Pro Ala Ala Gln Pro Thr
                530                 535                 540

Glu Gln Glu Ser Val Ser Leu Leu Cys Thr Ala Asp Arg Asn Thr Phe
            545                 550                 555

Glu Asn Leu Thr Trp Tyr Lys Leu Gly Ser Gln Ala Thr Ser Val His
        560                 565                 570

Met Gly Glu Ser Leu Thr Pro Val Cys Lys Asn Leu Asp Ala Leu Trp
    575                 580                 585

Lys Leu Asn Gly Thr Met Phe Ser Asn Ser Thr Asn Asp Ile Leu Ile
590                 595                 600                 605

Val Ala Phe Gln Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr Val Cys
```

```
                    610                 615                 620
Ser Ala Gln Asp Lys Lys Thr Lys Lys Arg His Cys Leu Val Lys Gln
                625                 630                 635

Leu Ile Ile Leu Glu Arg Met Ala Pro Met Ile Thr Gly Asn Leu Glu
            640                 645                 650

Asn Gln Thr Thr Thr Ile Gly Glu Thr Ile Glu Val Thr Cys Pro Ala
    655                 660                 665

Ser Gly Asn Pro Thr Pro His Ile Thr Trp Phe Lys Asp Asn Glu Thr
670                 675                 680                 685

Leu Val Glu Asp Ser Gly Ile Val Leu Arg Asp Gly Asn Arg Asn Leu
                690                 695                 700

Thr Ile Arg Arg Val Arg Lys Glu Asp Gly Gly Leu Tyr Thr Cys Gln
            705                 710                 715

Ala Cys Asn Val Leu Gly Cys Ala Arg Ala Glu Thr Leu Phe Ile Ile
        720                 725                 730

Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Val Ile Ile Leu Val Gly
    735                 740                 745

Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile Leu Val
750                 755                 760                 765

Arg Thr Val Lys Arg Ala Asn Glu Gly Glu Leu Lys Thr Gly Tyr Leu
                770                 775                 780

Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu Arg Cys Glu
            785                 790                 795

Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp Arg Leu
        800                 805                 810
```

```
Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val Ile Glu
    815                 820                 825

Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Lys Thr Val Ala
830                 835                 840                 845

Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg Ala Leu
                850                 855                 860

Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu Asn Val
            865                 870                 875

Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu Met Val
        880                 885                 890

Ile Val Glu Phe Ser Lys Phe Gly Asn Leu Ser Thr Tyr Leu Arg Gly
    895                 900                 905

Lys Arg Asn Glu Phe Val Pro Tyr Lys Ser Lys Gly Ala Arg Phe Arg
910                 915                 920                 925

Gln Gly Lys Asp Tyr Val Gly Glu Leu Ser Val Asp Leu Lys Arg Arg
                930                 935                 940

Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly Phe Val
            945                 950                 955

Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Ser Glu Glu
        960                 965                 970

Leu Tyr Lys Asp Phe Leu Thr Leu Glu His Leu Ile Cys Tyr Ser Phe
    975                 980                 985

Gln Val Ala Lys Gly Met Glu Phe Leu Ala Ser Arg Lys Cys Ile His
990                 995                 1000                1005

Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu Ser Glu Lys Asn Val Val
```

```
                    1010                1015                1020
Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Tyr Lys Asp Pro Asp
            1025                1030                1035
Tyr Val Arg Lys Gly Asp Ala Arg Leu Pro Leu Lys Trp Met Ala Pro
        1040                1045                1050
Glu Thr Ile Phe Asp Arg Val Tyr Thr Ile Gln Ser Asp Val Trp Ser
    1055                1060                1065
Phe Gly Val Leu Leu Trp Glu Ile Phe Ser Leu Gly Ala Ser Pro Tyr
1070                1075                1080                1085
Pro Gly Val Lys Ile Asp Glu Glu Phe Cys Arg Arg Leu Lys Glu Gly
                1090                1095                1100
Thr Arg Met Arg Ala Pro Asp Tyr Thr Thr Pro Glu Met Tyr Gln Thr
            1105                1110                1115
Met Leu Asp Cys Trp His Glu Asp Pro Asn Gln Arg Pro Ser Phe Ser
        1120                1125                1130
Glu Leu Val Glu His Leu Gly Asn Leu Leu Gln Ala Asn Ala Gln Gln
    1135                1140                1145
Asp Gly Lys Asp Tyr Ile Val Leu Pro Met Ser Glu Thr Leu Ser Met
1150                1155                1160                1165
Glu Glu Asp Ser Gly Leu Ser Leu Pro Thr Ser Pro Val Ser Cys Met
                1170                1175                1180
Glu Glu Glu Glu Val Cys Asp Pro Lys Phe His Tyr Asp Asn Thr Ala
            1185                1190                1195
Gly Ile Ser His Tyr Leu Gln Asn Ser Lys Arg Lys Ser Arg Pro Val
        1200                1205                1210
```

```
Ser Val Lys Thr Phe Glu Asp Ile Pro Leu Glu Glu Pro Glu Val Lys
    1215                1220                1225

Val Ile Pro Asp Asp Ser Gln Thr Asp Ser Gly Met Val Leu Ala Ser
1230                1235                1240                1245

Glu Glu Leu Lys Thr Leu Glu Asp Arg Asn Lys Leu Ser Pro Ser Phe
                1250                1255                1260

Gly Gly Met Met Pro Ser Lys Ser Arg Glu Ser Val Ala Ser Glu Gly
            1265                1270                1275

Ser Asn Gln Thr Ser Gly Tyr Gln Ser Gly Tyr His Ser Asp Asp Thr
        1280                1285                1290

Asp Thr Thr Val Tyr Ser Ser Asp Glu Ala Gly Leu Leu Lys Met Val
    1295                1300                1305

Asp Ala Ala Val His Ala Asp Ser Gly Thr Thr Leu Gln Leu Thr Ser
1310                1315                1320                1325

Cys Leu Asn Gly Ser Gly Pro Val Pro Ala Pro Pro Pro Thr Pro Gly
                1330                1335                1340

Asn His Glu Arg Gly Ala Ala
            1345
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AATTCGTCGA CTTTCTGTCA CCATGAGTGC ACTTCTGATC CTAGCCCTTG TGGGAGCTGC 60

TGTTGCTGAC TACAAAGATG ATGATGACAA GATCTA 96

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCTTAGATC TTGTCATCAT CATCTTTGTA GTCAGCAACA GCAGCTCCCA CAGAGGCTAG 60

GATCAGAAGT GCACTCATGG TGACAGAAAG TCGACG 96

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAAGATC TCAAACCAAG ACCTGCCTGT 30

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCAATGGCGG CCGCTCAGGA GATGTTGTCT TGGA 34

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Ala Gln Ser Leu Ser Phe Xaa Phe Thr Lys Phe Asp Leu Asp
1               5                   10
```

CLAIMS

What is claimed is:

1. A protein that binds to the Flk2 receptor comprising the amino acid sequence AQSLSFXFTKFDLD shown in SEQ. ID. NO. 11, wherein X is any amino acid.

Fig. 1a.1

```
GCGGCCTGGC TACCGCGCGC TCCGGAGGCC ATG CGG GCG TTG GCG CAG CGC AGC
                                 Met Arg Ala Leu Ala Gln Arg Ser
                                 -27     -25                 -20

GAC CGG CGG CTG CTG CTG CTT GTT GTT TTG TCA GTA ATG ATT CTT GAG
Asp Arg Arg Leu Leu Leu Leu Val Val Leu Ser Val Met Ile Leu Glu
                -15                 -10                 -5

ACC GTT ACA AAC CAA GAC CTG CCT GTG ATC AAG TGT GTT TTA ATC AGT
Thr Val Thr Asn Gln Asp Leu Pro Val Ile Lys Cys Val Leu Ile Ser
              1               5                 10

CAT GAG AAC AAT GGC TCA TCA GCG GGA AAG CCA TCA TCG TAC CGA ATG
His Glu Asn Asn Gly Ser Ser Ala Gly Lys Pro Ser Ser Tyr Arg Met
     15                  20                  25

GTG CGA GGA TCC CCA GAA GAC CTC CAG TGT ACC CCG AGG CGC CAG AGT
Val Arg Gly Ser Pro Glu Asp Leu Gln Cys Thr Pro Arg Arg Gln Ser
 30                  35                  40                  45

GAA GGG ACG GTA TAT GAA GCG GCC ACC GTG GAG GTG GCC GAG TCT GGG
Glu Gly Thr Val Tyr Glu Ala Ala Thr Val Glu Val Ala Glu Ser Gly
                 50                  55                  60

TCC ATC ACC CTG CAA GTG CAG CTC GCC ACC CCA GGG GAC CTT TCC TGC
Ser Ile Thr Leu Gln Val Gln Leu Ala Thr Pro Gly Asp Leu Ser Cys
             65                  70                  75

CTC TGG GTC TTT AAG CAC AGC TCC CTG GGC TGC CAG CCG CAC TTT GAT
Leu Trp Val Phe Lys His Ser Ser Leu Gly Cys Gln Pro His Phe Asp
         80                  85                  90

TTA CAA AAC AGA GGA ATC GTT TCC ATG GCC ATC TTG AAC GTG ACA GAG
Leu Gln Asn Arg Gly Ile Val Ser Met Ala Ile Leu Asn Val Thr Glu
     95                 100                 105

ACC CAG GCA GGA GAA TAC CTA CTC CAT ATT CAG AGC GAA CGC GCC AAC
Thr Gln Ala Gly Glu Tyr Leu Leu His Ile Gln Ser Glu Arg Ala Asn
110                 115                 120                 125

TAC ACA GTA CTG TTC ACA GTG AAT GTA AGA GAT ACA CAG CTG TAT GTG
Tyr Thr Val Leu Phe Thr Val Asn Val Arg Asp Thr Gln Leu Tyr Val
                130                 135                 140

CTA AGG AGA CCT TAC TTT AGG AAG ATG GAA AAC CAG GAT GCA CTG CTC
Leu Arg Arg Pro Tyr Phe Arg Lys Met Glu Asn Gln Asp Ala Leu Leu
            145                 150                 155
```


SUBSTITUTE SHEET (RULE 26)

Fig. 1a.2

```
TGC ATC TCC GAG GGT GTT CCG GAG CCC ACT GTG GAG TGG GTG CTC TGC
Cys Ile Ser Glu Gly Val Pro Glu Pro Thr Val Glu Trp Val Leu Cys
        160                 165                 170

AGC TCC CAC AGG GAA AGC TGT AAA GAA GAA GGC CCT GCT GTT GTC AGA
Ser Ser His Arg Glu Ser Cys Lys Glu Glu Gly Pro Ala Val Val Arg
    175                 180                 185

AAG GAG GAA AAG GTA CTT CAT GAG TTG TTC GGA ACA GAC ATC AGA TGC
Lys Glu Glu Lys Val Leu His Glu Leu Phe Gly Thr Asp Ile Arg Cys
190                 195                 200                 205

TGT GCT AGA AAT GCA CTG GGC CGC GAA TGC ACC AAG CTG TTC ACC ATA
Cys Ala Arg Asn Ala Leu Gly Arg Glu Cys Thr Lys Leu Phe Thr Ile
                210                 215                 220

GAT CTA AAC CAG GCT CCT CAG AGC ACA CTG CCC CAG TTA TTC CTG AAA
Asp Leu Asn Gln Ala Pro Gln Ser Thr Leu Pro Gln Leu Phe Leu Lys
            225                 230                 235

GTG GGG GAA CCC TTG TGG ATC AGG TGT AAG GCC ATC CAT GTG AAC CAT
Val Gly Glu Pro Leu Trp Ile Arg Cys Lys Ala Ile His Val Asn His
        240                 245                 250

GGA TTC GGG CTC ACC TGG GAG CTG GAA GAC AAA GCC CTG GAG GAG GGC
Gly Phe Gly Leu Thr Trp Glu Leu Glu Asp Lys Ala Leu Glu Glu Gly
    255                 260                 265

AGC TAC TTT GAG ATG AGT ACC TAC TCC ACA AAC AGG ACC ATG ATT CGG
Ser Tyr Phe Glu Met Ser Thr Tyr Ser Thr Asn Arg Thr Met Ile Arg
270                 275                 280                 285

ATT CTC TTG GCC TTT GTG TCT TCC GTG GGA AGG AAC GAC ACC GGA TAT
Ile Leu Leu Ala Phe Val Ser Ser Val Gly Arg Asn Asp Thr Gly Tyr
                290                 295                 300

TAC ACC TGC TCT TCC TCA AAG CAC CCC AGC CAG TCA GCG TTG GTG ACC
Tyr Thr Cys Ser Ser Ser Lys His Pro Ser Gln Ser Ala Leu Val Thr
            305                 310                 315

ATC CTA GAA AAA GGG TTT ATA AAC GCT ACC AGC TCG CAA GAA GAG TAT
Ile Leu Glu Lys Gly Phe Ile Asn Ala Thr Ser Ser Gln Glu Glu Tyr
        320                 325                 330

GAA ATT GAC CCG TAC GAA AAG TTC TGC TTC TCA GTC AGG TTT AAA GCG
Glu Ile Asp Pro Tyr Glu Lys Phe Cys Phe Ser Val Arg Phe Lys Ala
    335                 340                 345
```

Fig. 1a.3

```
TAC CCA CGA ATC CGA TGC ACG TGG ATC TTC TCT CAA GCC TCA TTT CCT
Tyr Pro Arg Ile Arg Cys Thr Trp Ile Phe Ser Gln Ala Ser Phe Pro
350                 355                 360                 365

TGT GAA CAG AGA GGC CTG GAG GAT GGG TAC AGC ATA TCT AAA TTT TGC
Cys Glu Gln Arg Gly Leu Glu Asp Gly Tyr Ser Ile Ser Lys Phe Cys
                370                 375                 380

GAT CAT AAG AAC AAG CCA GGA GAG TAC ATA TTC TAT GCA GAA AAT GAT
Asp His Lys Asn Lys Pro Gly Glu Tyr Ile Phe Tyr Ala Glu Asn Asp
            385                 390                 395

GAC GCC CAG TTC ACC AAA ATG TTC ACG CTG AAT ATA AGA AAG AAA CCT
Asp Ala Gln Phe Thr Lys Met Phe Thr Leu Asn Ile Arg Lys Lys Pro
        400                 405                 410

CAA GTG CTA GCA AAT GCC TCA GCC AGC CAG GCG TCC TGT TCC TCT GAT
Gln Val Leu Ala Asn Ala Ser Ala Ser Gln Ala Ser Cys Ser Ser Asp
    415                 420                 425

GGC TAC CCG CTA CCC TCT TGG ACC TGG AAG AAG TGT TCG GAC AAA TCT
Gly Tyr Pro Leu Pro Ser Trp Thr Trp Lys Lys Cys Ser Asp Lys Ser
430                 435                 440                 445

CCC AAT TGC ACG GAG GAA ATC CCA GAA GGA GTT TGG AAT AAA AAG GCT
Pro Asn Cys Thr Glu Glu Ile Pro Glu Gly Val Trp Asn Lys Lys Ala
                450                 455                 460

AAC AGA AAA GTG TTT GGC CAG TGG GTG TCG AGC AGT ACT CTA AAT ATG
Asn Arg Lys Val Phe Gly Gln Trp Val Ser Ser Ser Thr Leu Asn Met
            465                 470                 475

AGT GAG GCC GGG AAA GGG CTT CTG GTC AAA TGC TGT GCG TAC AAT TCT
Ser Glu Ala Gly Lys Gly Leu Leu Val Lys Cys Cys Ala Tyr Asn Ser
        480                 485                 490

ATG GGC ACG TCT TGC GAA ACC ATC TTT TTA AAC TCA CCA GGC CCC TTC
Met Gly Thr Ser Cys Glu Thr Ile Phe Leu Asn Ser Pro Gly Pro Phe
    495                 500                 505

CCT TTC ATC CAA GAC AAC ATC TCC TTC TAT GCG ACC ATT GGG CTC TGT
Pro Phe Ile Gln Asp Asn Ile Ser Phe Tyr Ala Thr Ile Gly Leu Cys
510                 515                 520                 525

CTC CCC TTC ATT GTT GTT CTC ATT GTG TTG ATC TGC CAC AAA TAC AAA
Leu Pro Phe Ile Val Val Leu Ile Val Leu Ile Cys His Lys Tyr Lys
                530                 535                 540
```

Fig. 1a.4

```
AAG CAA TTT AGG TAC GAG AGT CAG CTG CAG ATG ATC CAG GTG ACT GGC
Lys Gln Phe Arg Tyr Glu Ser Gln Leu Gln Met Ile Gln Val Thr Gly
            545                 550                 555

CCC CTG GAT AAC GAG TAC TTC TAC GTT GAC TTC AGG GAC TAT GAA TAT
Pro Leu Asp Asn Glu Tyr Phe Tyr Val Asp Phe Arg Asp Tyr Glu Tyr
        560                 565                 570

GAC CTT AAG TGG GAG TTC CCG AGA GAG AAC TTA GAG TTT GGG AAG GTC
Asp Leu Lys Trp Glu Phe Pro Arg Glu Asn Leu Glu Phe Gly Lys Val
    575                 580                 585

CTG GGG TCT GGC GCT TTC GGG AGG GTG ATG AAC GCC ACG GCC TAT GGC
Leu Gly Ser Gly Ala Phe Gly Arg Val Met Asn Ala Thr Ala Tyr Gly
590                 595                 600                 605

ATT AGT AAA ACG GGA GTC TCA ATT CAG GTG GCG GTG AAG ATG CTA AAA
Ile Ser Lys Thr Gly Val Ser Ile Gln Val Ala Val Lys Met Leu Lys
                610                 615                 620

GAG AAA GCT GAC AGC TGT GAA AAA GAA GCT CTC ATG TCG GAG CTC AAA
Glu Lys Ala Asp Ser Cys Glu Lys Glu Ala Leu Met Ser Glu Leu Lys
            625                 630                 635

ATG ATG ACC CAC CTG GGA CAC CAT GAC AAC ATC GTG AAT CTG CTG GGG
Met Met Thr His Leu Gly His His Asp Asn Ile Val Asn Leu Leu Gly
        640                 645                 650

GCA TGC ACA CTG TCA GGG CCA GTG TAC TTG ATT TTT GAA TAT TGT TGC
Ala Cys Thr Leu Ser Gly Pro Val Tyr Leu Ile Phe Glu Tyr Cys Cys
    655                 660                 665

TAT GGT GAC CTC CTC AAC TAC CTA AGA AGT AAA AGA GAG AAG TTT CAC
Tyr Gly Asp Leu Leu Asn Tyr Leu Arg Ser Lys Arg Glu Lys Phe His
670                 675                 680                 685

AGG ACA TGG ACA GAG ATT TTT AAG GAA CAT AAT TTC AGT TCT TAC CCT
Arg Thr Trp Thr Glu Ile Phe Lys Glu His Asn Phe Ser Ser Tyr Pro
                690                 695                 700

ACT TTC CAG GCA CAT TCA AAT TCC AGC ATG CCT GGT TCA CGA GAA GTT
Thr Phe Gln Ala His Ser Asn Ser Ser Met Pro Gly Ser Arg Glu Val
            705                 710                 715

CAG TTA CAC CCG CCC TTG GAT CAG CTC TCA GGG TTC AAT GGG AAT TCA
Gln Leu His Pro Pro Leu Asp Gln Leu Ser Gly Phe Asn Gly Asn Ser
        720                 725                 730
```

Fig. 1a.5

```
ATT CAT TCT GAA GAT GAG ATT GAA TAT GAA AAC CAG AAG AGG CTG GCA
Ile His Ser Glu Asp Glu Ile Glu Tyr Glu Asn Gln Lys Arg Leu Ala
    735                 740                 745

GAA GAA GAG GAG GAA GAT TTG AAC GTG CTG ACG TTT GAA GAC CTC CTT
Glu Glu Glu Glu Glu Asp Leu Asn Val Leu Thr Phe Glu Asp Leu Leu
750                 755                 760                 765

TGC TTT GCG TAC CAA GTG GCC AAA GGC ATG GAA TTC CTG GAG TTC AAG
Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu Phe Leu Glu Phe Lys
                770                 775                 780

TCG TGT GTC CAC AGA GAC CTG GCA GCC AGG AAT GTG TTG GTC ACC CAC
Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr His
            785                 790                 795

GGG AAG GTG GTG AAG ATC TGT GAC TTT GGA CTG GCC CGA GAC ATC CTG
Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Leu
        800                 805                 810

AGC GAC TCC AGC TAC GTC GTC AGG GGC AAC GCA CGG CTG CCG GTG AAG
Ser Asp Ser Ser Tyr Val Val Arg Gly Asn Ala Arg Leu Pro Val Lys
    815                 820                 825

TGG ATG GCA CCC GAG AGC TTA TTT GAA GGG ATC TAC ACA ATC AAG AGT
Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile Tyr Thr Ile Lys Ser
830                 835                 840                 845

GAC GTC TGG TCC TAC GGC ATC CTT CTC TGG GAG ATA TTT TCA CTG GGT
Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu Gly
                850                 855                 860

GTG AAC CCT TAC CCT GGC ATT CCT GTC GAC GCT AAC TTC TAT AAA CTG
Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala Asn Phe Tyr Lys Leu
            865                 870                 875

ATT CAG AGT GGA TTT AAA ATG GAG CAG CCA TTC TAT GCC ACA GAA GGG
Ile Gln Ser Gly Phe Lys Met Glu Gln Pro Phe Tyr Ala Thr Glu Gly
        880                 885                 890

ATA TAC TTT GTA ATG CAA TCC TGC TGG GCT TTT GAC TCA AGG AAG CGG
Ile Tyr Phe Val Met Gln Ser Cys Trp Ala Phe Asp Ser Arg Lys Arg
    895                 900                 905
```

Fig. 1a.6

```
CCA TCC TTC CCC AAC CTG ACT TCA TTT TTA GGA TGT CAG CTG GCA GAG
Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly Cys Gln Leu Ala Glu
910                 915                 920                 925

GCA GAA GAA GCA TGT ATC AGA ACA TCC ATC CAT CTA CCA AAA CAG GCG
Ala Glu Glu Ala Cys Ile Arg Thr Ser Ile His Leu Pro Lys Gln Ala
                930                 935                 940

GCC CCT CAG CAG AGA GGC GGG CTC AGA GCC CAG TCG CCA CAG CGC CAG
Ala Pro Gln Gln Arg Gly Gly Leu Arg Ala Gln Ser Pro Gln Arg Gln
            945                 950                 955

GTG AAG ATT CAC AGA GAA AGA AGT TAGCGAGGAG GCCTTGGACC CCGCCACCCT
Val Lys Ile His Arg Glu Arg Ser
        960                 965

AGCAGGCTGT AGACCGCAGA GCCAAGATTA GCCTCGCCTC TGAGGAAGCG CCCTACAGCG

CGTTGCTTCG CTGGACTTTT CTCTAGATGC TGTCTGCCAT TACTCCAAAG TGACTTCTAT

AAAATCAAAC CTCTCCTCGC ACAGGCGGGA GAGCCAATAA TGAGACTTGT TGGTGAGCCC

GCCTACCCTG GGGGCCTTTC CACGAGCTTG AGGGGAAAGC CATGTATCTG AAATATAGTA

TATTCTTGTA AATACGTGAA ACAAACCAAA CCCGTTTTTT GCTAAGGGAA AGCTAAATAT

GATTTTTAAA AATCTATGTT TTAAAATACT ATGTAACTTT TTCATCTATT TAGTGATATA

TTTTATGGAT GGAAATAAAC TTTCTACTGT AAAAAAAAAA AAAAAAAAAA AAAAAAA
```

Fig. 1b.1

```
CGAGGCGGCA TCCGAGGGCT GGGCCGGCGC CCTGGGGGAC CCCGGGCTCC GGAGGCC

ATG CCG GCG TTG GCG CGC GAC GCG GGC ACC GTG CCG CTG CTC GTT GTT
Met Pro Ala Leu Ala Arg Asp Ala Gly Thr Val Pro Leu Leu Val Val
-27     -25                 -20                 -15

TTT TCT GCA ATG ATA TTT GGG ACT ATT ACA AAT CAA GAT CTG CCT GTG
Phe Ser Ala Met Ile Phe Gly Thr Ile Thr Asn Gln Asp Leu Pro Val
    -10                  -5                   1                5

ATC AAG TGT GTT TTA ATC AAT CAT AAG AAC AAT GAT TCA TCA GTG GGG
Ile Lys Cys Val Leu Ile Asn His Lys Asn Asn Asp Ser Ser Val Gly
                 10                  15                  20

AAG TCA TCA TCA TAT CCC ATG GTA TCA GAA TCC CCG GAA GAC CTC GGG
Lys Ser Ser Ser Tyr Pro Met Val Ser Glu Ser Pro Glu Asp Leu Gly
             25                  30                  35

TGT GCG TTG AGA CCC CAG AGC TCA GGG ACA GTG TAC GAA GCT GCC GCT
Cys Ala Leu Arg Pro Gln Ser Ser Gly Thr Val Tyr Glu Ala Ala Ala
         40                  45                  50

GTG GAA GTG GAT GTA TCT GCT TCC ATC ACA CTG CAA GTG CTG GTC GAT
Val Glu Val Asp Val Ser Ala Ser Ile Thr Leu Gln Val Leu Val Asp
     55                  60                  65

GCC CCA GGG AAC ATT TCC TGT CTC TGG GTC TTT AAG CAC AGC TCC CTG
Ala Pro Gly Asn Ile Ser Cys Leu Trp Val Phe Lys His Ser Ser Leu
 70                  75                  80                  85

AAT TGC CAG CCA CAT TTT GAT TTA CAA AAC AGA GGA GTT GTT TCC ATG
Asn Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Val Val Ser Met
                 90                  95                 100

GTC ATT TTG AAA ATG ACA GAA ACC CAA GCT GGA GAA TAC CTA CTT TTT
Val Ile Leu Lys Met Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu Phe
            105                 110                 115

ATT CAG AGT GAA GCT ACC AAT TAC ACA ATA TTG TTT ACA GTG AGT ATA
Ile Gln Ser Glu Ala Thr Asn Tyr Thr Ile Leu Phe Thr Val Ser Ile
        120                 125                 130

AGA AAT ACC CTG CTT TAC ACA TTA AGA AGA CCT TAC TTT AGA AAA ATG
Arg Asn Thr Leu Leu Tyr Thr Leu Arg Arg Pro Tyr Phe Arg Lys Met
    135                 140                 145
```

Fig. 1b.2

GAA	AAC	CAG	GAC	GCC	CTG	GTC	TGC	ATA	TCT	GAG	AGC	GTT	CCA	GAG	CCG
Glu	Asn	Gln	Asp	Ala	Leu	Val	Cys	Ile	Ser	Glu	Ser	Val	Pro	Glu	Pro
150					155					160					165
ATC	GTG	GAA	TGG	GTG	CTT	TGC	GAT	TCA	CAG	GGG	GAA	AGC	TGT	AAA	GAA
Ile	Val	Glu	Trp	Val	Leu	Cys	Asp	Ser	Gln	Gly	Glu	Ser	Cys	Lys	Glu
				170					175					180	
GAA	AGT	CCA	GCT	GTT	GTT	AAA	AAG	GAG	GAA	AAA	GTG	CTT	CAT	GAA	TTA
Glu	Ser	Pro	Ala	Val	Val	Lys	Lys	Glu	Glu	Lys	Val	Leu	His	Glu	Leu
			185					190					195		
TTT	GGG	ACG	GAC	ATA	AGG	TGC	TGT	GCC	AGA	AAT	GAA	CTG	GGC	AGG	GAA
Phe	Gly	Thr	Asp	Ile	Arg	Cys	Cys	Ala	Arg	Asn	Glu	Leu	Gly	Arg	Glu
		200					205					210			
TGC	ACC	AGG	CTG	TTC	ACA	ATA	GAT	CTA	AAT	CAA	ACT	CCT	CAG	ACC	ACA
Cys	Thr	Arg	Leu	Phe	Thr	Ile	Asp	Leu	Asn	Gln	Thr	Pro	Gln	Thr	Thr
	215					220					225				
TTG	CCA	CAA	TTA	TTT	CTT	AAA	GTA	GGG	GAA	CCC	TTA	TGG	ATA	AGG	TGC
Leu	Pro	Gln	Leu	Phe	Leu	Lys	Val	Gly	Glu	Pro	Leu	Trp	Ile	Arg	Cys
230					235					240					245
AAA	GCT	GTT	CAT	GTG	AAC	CAT	GGA	TTC	GGG	CTC	ACC	TGG	GAA	TTA	GAA
Lys	Ala	Val	His	Val	Asn	His	Gly	Phe	Gly	Leu	Thr	Trp	Glu	Leu	Glu
				250					255					260	
AAC	AAA	GCA	CTC	GAG	GAG	GGC	AAC	TAC	TTT	GAG	ATG	AGT	ACC	TAT	TCA
Asn	Lys	Ala	Leu	Glu	Glu	Gly	Asn	Tyr	Phe	Glu	Met	Ser	Thr	Tyr	Ser
			265					270					275		
ACA	AAC	AGA	ACT	ATG	ATA	CGG	ATT	CTG	TTT	GCT	TTT	GTA	TCA	TCA	GTG
Thr	Asn	Arg	Thr	Met	Ile	Arg	Ile	Leu	Phe	Ala	Phe	Val	Ser	Ser	Val
		280					285					290			
GCA	AGA	AAC	GAC	ACC	GGA	TAC	TAC	ACT	TGT	TCC	TCT	TCA	AAG	CAT	CCC
Ala	Arg	Asn	Asp	Thr	Gly	Tyr	Tyr	Thr	Cys	Ser	Ser	Ser	Lys	His	Pro
	295					300					305				
AGT	CAA	TCA	GCT	TTG	GTT	ACC	ATC	GTA	GGA	AAG	GGA	TTT	ATA	AAT	GCT
Ser	Gln	Ser	Ala	Leu	Val	Thr	Ile	Val	Gly	Lys	Gly	Phe	Ile	Asn	Ala
310					315					320					325
ACC	AAT	TCA	AGT	GAA	GAT	TAT	GAA	ATT	GAC	CAA	TAT	GAA	GAG	TTT	TGT
Thr	Asn	Ser	Ser	Glu	Asp	Tyr	Glu	Ile	Asp	Gln	Tyr	Glu	Glu	Phe	Cys
				330					335					340	

Fig. 1b.3

```
TTT TCT GTC AGG TTT AAA GCC TAC CCA CAA ATC AGA TGT ACG TGG ACC
Phe Ser Val Arg Phe Lys Ala Tyr Pro Gln Ile Arg Cys Thr Trp Thr
            345                 350                 355

TTC TCT CGA AAA TCA TTT CCT TGT GAG CAA AAG GGT CTT GAT AAC GGA
Phe Ser Arg Lys Ser Phe Pro Cys Glu Gln Lys Gly Leu Asp Asn Gly
        360                 365                 370

TAC AGC ATA TCC AAG TTT TGC AAT CAT AAG CAC CAG CCA GGA GAA TAT
Tyr Ser Ile Ser Lys Phe Cys Asn His Lys His Gln Pro Gly Glu Tyr
    375                 380                 385

ATA TTC CAT GCA GAA AAT GAT GAT GCC CAA TTT ACC AAA ATG TTC ACG
Ile Phe His Ala Glu Asn Asp Asp Ala Gln Phe Thr Lys Met Phe Thr
390                 395                 400                 405

CTG AAT ATA AGA AGG AAA CCT CAA GTG CTC GCA GAA GCA TCG GCA AGT
Leu Asn Ile Arg Arg Lys Pro Gln Val Leu Ala Glu Ala Ser Ala Ser
                410                 415                 420

CAG GCG TCC TGT TTC TCG GAT GGA TAC CCA TTA CCA TCT TGG ACC TGG
Gln Ala Ser Cys Phe Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr Trp
            425                 430                 435

AAG AAG TGT TCA GAC AAG TCT CCC AAC TGC ACA GAA GAG ATC ACA GAA
Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Thr Glu
        440                 445                 450

GGA GTC TGG AAT AGA AAG GCT AAC AGA AAA GTG TTT GGA CAG TGG GTG
Gly Val Trp Asn Arg Lys Ala Asn Arg Lys Val Phe Gly Gln Trp Val
    455                 460                 465

TCG AGC AGT ACT CTA AAC ATG AGT GAA GCC ATA AAA GGG TTC CTG GTC
Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Ile Lys Gly Phe Leu Val
470                 475                 480                 485

AAG TGC TGT GCA TAC AAT TCC CTT GGC ACA TCT TGT GAG ACG ATC CTT
Lys Cys Cys Ala Tyr Asn Ser Leu Gly Thr Ser Cys Glu Thr Ile Leu
                490                 495                 500

TTA AAC TCT CCA GGC CCC TTC CCT TTC ATC CAA GAC AAC ATC TCA TTC
Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser Phe
            505                 510                 515

TAT GCA ACA ATT GGT GTT TGT CTC CTC TTC ATT GTC GTT TTA ACC CTG
Tyr Ala Thr Ile Gly Val Cys Leu Leu Phe Ile Val Val Leu Thr Leu
        520                 525                 530
```


SUBSTITUTE SHEET (RULE 26)

Fig. 1b.4

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CTA ATT TGT CAC AAG TAC AAA AAG CAA TTT AGG TAT GAA AGC CAG CTA
Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln Leu
    535                 540                 545

CAG ATG GTA CAG GTG ACC GGC TCC TCA GAT AAT GAG TAC TTC TAC GTT
Gln Met Val Gln Val Thr Gly Ser Ser Asp Asn Glu Tyr Phe Tyr Val
550                 555                 560                 565

GAT TTC AGA GAA TAT GAA TAT GAT CTC AAA TGG GAG TTT CCA AGA GAA
Asp Phe Arg Glu Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg Glu
                570                 575                 580

AAT TTA GAG TTT GGG AAG GTA CTA GGA TCA GGT GCT TTT GGA AAA GTG
Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Lys Val
            585                 590                 595

ATG AAC GCA ACA GCT TAT GGA ATT AGC AAA ACA GGA GTC TCA ATC CAG
Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile Gln
        600                 605                 610

GTT GCC GTC AAA ATG CTG AAA GAA AAA GCA GAC AGC TCT GAA AGA GAG
Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Ser Glu Arg Glu
    615                 620                 625

GCA CTC ATG TCA GAA CTC AAG ATG ATG ACC CAG CTG GGA AGC CAC GAG
Ala Leu Met Ser Glu Leu Lys Met Met Thr Gln Leu Gly Ser His Glu
630                 635                 640                 645

AAT ATT GTG AAC CTG CTG GGG GCG TGC ACA CTG TCA GGA CCA ATT TAC
Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Ile Tyr
                650                 655                 660

TTG ATT TTT GAA TAC TGT TGC TAT GGT GAT CTT CTC AAC TAT CTA AGA
Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu Arg
            665                 670                 675

AGT AAA AGA GAA AAA TTT CAC AGG ACT TGG ACA GAG ATT TTC AAG GAA
Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys Glu
        680                 685                 690

CAC AAT TTC AGT TTT TAC CCC ACT TTC CAA TCA CAT CCA AAT TCC AGC
His Asn Phe Ser Phe Tyr Pro Thr Phe Gln Ser His Pro Asn Ser Ser
    695                 700                 705

ATG CCT GGT TCA AGA GAA GTT CAG ATA CAC CCG GAC TCG GAT CAA ATC
Met Pro Gly Ser Arg Glu Val Gln Ile His Pro Asp Ser Asp Gln Ile
710                 715                 720                 725
```

Fig. 1b.5

TCA	GGG	CTT	CAT	GGG	AAT	TCA	TTT	CAC	TCT	GAA	GAT	GAA	ATT	GAA	TAT
Ser	Gly	Leu	His	Gly	Asn	Ser	Phe	His	Ser	Glu	Asp	Glu	Ile	Glu	Tyr
				730					735					740	
GAA	AAC	CAA	AAA	AGG	CTG	GAA	GAA	GAG	GAG	GAC	TTG	AAT	GTG	CTT	ACA
Glu	Asn	Gln	Lys	Arg	Leu	Glu	Glu	Glu	Glu	Asp	Leu	Asn	Val	Leu	Thr
			745					750					755		
TTT	GAA	GAT	CTT	CTT	TGC	TTT	GCA	TAT	CAA	GTT	GCC	AAA	GGA	ATG	GAA
Phe	Glu	Asp	Leu	Leu	Cys	Phe	Ala	Tyr	Gln	Val	Ala	Lys	Gly	Met	Glu
		760					765					770			
TTT	CTG	GAA	TTT	AAG	TCG	TGT	GTT	CAC	AGA	GAC	CTG	GCC	GCC	AGG	AAC
Phe	Leu	Glu	Phe	Lys	Ser	Cys	Val	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn
	775					780					785				
GTG	CTT	GTC	ACC	CAC	GGG	AAA	GTG	GTG	AAG	ATA	TGT	GAC	TTT	GGA	TTG
Val	Leu	Val	Thr	His	Gly	Lys	Val	Val	Lys	Ile	Cys	Asp	Phe	Gly	Leu
790					795					800					805
GCT	CGA	GAT	ATC	ATG	AGT	GAT	TCC	AAC	TAT	GTT	GTC	AGG	GGC	AAT	GCC
Ala	Arg	Asp	Ile	Met	Ser	Asp	Ser	Asn	Tyr	Val	Val	Arg	Gly	Asn	Ala
				810					815					820	
CGT	CTG	CCT	GTA	AAA	TGG	ATG	GCC	CCC	GAA	AGC	CTG	TTT	GAA	GGC	ATC
Arg	Leu	Pro	Val	Lys	Trp	Met	Ala	Pro	Glu	Ser	Leu	Phe	Glu	Gly	Ile
			825					830					835		
TAC	ACC	ATT	AAG	AGT	GAT	GTC	TGG	TCA	TAT	GGA	ATA	TTA	CTG	TGG	GAA
Tyr	Thr	Ile	Lys	Ser	Asp	Val	Trp	Ser	Tyr	Gly	Ile	Leu	Leu	Trp	Glu
		840					845					850			
ATC	TTC	TCA	CTT	GGT	GTG	AAT	CCT	TAC	CCT	GGC	ATT	CCG	GTT	GAT	GCT
Ile	Phe	Ser	Leu	Gly	Val	Asn	Pro	Tyr	Pro	Gly	Ile	Pro	Val	Asp	Ala
	855					860					865				
AAC	TTC	TAC	AAA	CTG	ATT	CAA	AAT	GGA	TTT	AAA	ATG	GAT	CAG	CCA	TTT
Asn	Phe	Tyr	Lys	Leu	Ile	Gln	Asn	Gly	Phe	Lys	Met	Asp	Gln	Pro	Phe
870					875					880					885
TAT	GCT	ACA	GAA	GAA	ATA	TAC	ATT	ATA	ATG	CAA	TCC	TGC	TGG	GCT	TTT
Tyr	Ala	Thr	Glu	Glu	Ile	Tyr	Ile	Ile	Met	Gln	Ser	Cys	Trp	Ala	Phe
				890					895					900	
GAC	TCA	AGG	AAA	CGG	CCA	TCC	TTC	CCT	AAT	TTG	ACT	TCG	TTT	TTA	GGA
Asp	Ser	Arg	Lys	Arg	Pro	Ser	Phe	Pro	Asn	Leu	Thr	Ser	Phe	Leu	Gly
			905					910					915		

Fig. 1b.6

```
TGT CAG CTG GCA GAT GCA GAA GAA GCG ATG TAT CAG AAT GTG GAT GGC
Cys Gln Leu Ala Asp Ala Glu Glu Ala Met Tyr Gln Asn Val Asp Gly
        920                 925                 930

CGT GTT TCG GAA TGT CCT CAC ACC TAC CAA AAC AGG CGA CCT TTC AGC
Arg Val Ser Glu Cys Pro His Thr Tyr Gln Asn Arg Arg Pro Phe Ser
    935                 940                 945

AGA GAG ATG GAT TTG GGG CTA CTC TCT CCG CAG GCT CAG GTC GAA GAT
Arg Glu Met Asp Leu Gly Leu Leu Ser Pro Gln Ala Gln Val Glu Asp
950                 955                 960                 965

TCG TAGAGGAACA ATTTAGTTTT AAGGACTTCA TCCCTCCACC TATCCCTAAC
Ser

AGGCTGTAGA TTACCAAAAC AAGATTAATT TCATCACTAA AAGAAAATCT ATTATCAACT

GCTGCTTCAC CAGACTTTTC TCTAGAAGCC GTCTGCGTTT ACTCTTGTTT TCAAAGGGAC

TTTTGTAAAA TCAAATCATC CTGTCACAAG GCAGGAGGAG CTGATAATGA ACTTTATTGG

AGCATTGATC TGCATCCAAG GCCTTCTCAG GCCGGCTTGA GTGAATTGTG TACCTGAAGT

ACAGTATATT CTTGTAAATA CATAAAACAA AAGCATTTTG CTAAGGAGAA GCTAATATGA

TTTTTTAAGT CTATGTTTTA AAATAATATG TAAATTTTTC AGCTATTTAG TGATATATTT

TATGGGTGGG AATAAAATTT CTACTACAGA AAAAAAAAAA AAAAAAAAAA AAAAA
```

Fig. 2.1

```
CTGTGTCCCG CAGCCGGATA ACCTGGCTGA CCCGATTCCG CGGACACCCG TGCAGCCGCG

GCTGGAGCCA GGGCGCCGGT GCCCGCGCTC TCCCCGGTCT TGCGCTGCGG GGGCCGATAC

CGCCTCTGTG ACTTCTTTGC GGGCCAGGGA CGGAGAAGGA GTCTGTGCCT GAGAAACTGG

GCTCTGTGCC CAGGCGCGAG GTGCAGG    ATG GAG AGC AAG GGC CTG CTA GCT
                                 Met Glu Ser Lys Gly Leu Leu Ala
                                -19             -15

GTC GCT CTG TGG TTC TGC GTG GAG ACC CGA GCC GCC TCT GTG GGT TTG
Val Ala Leu Trp Phe Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu
    -10                 -5                  1               5

CCT GGC GAT TTT CTC CAT CCC CCC AAG CTC AGC ACA CAG AAA GAC ATA
Pro Gly Asp Phe Leu His Pro Pro Lys Leu Ser Thr Gln Lys Asp Ile
                10                  15                  20

CTG ACA ATT TTG GCA AAT ACA ACC CTT CAG ATT ACT TGC AGG GGA CAG
Leu Thr Ile Leu Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln
            25                  30                  35

CGG GAC CTG GAC TGG CTT TGG CCC AAT GCT CAG CGT GAT TCT GAG GAA
Arg Asp Leu Asp Trp Leu Trp Pro Asn Ala Gln Arg Asp Ser Glu Glu
        40                  45                  50

AGG GTA TTG GTG ACT GAA TGC GGC GGT GGT GAC AGT ATC TTC TGC AAA
Arg Val Leu Val Thr Glu Cys Gly Gly Gly Asp Ser Ile Phe Cys Lys
    55                  60                  65

ACA CTC ACC ATT CCC AGG GTG GTT GGA AAT GAT ACT GGA GCC TAC AAG
Thr Leu Thr Ile Pro Arg Val Val Gly Asn Asp Thr Gly Ala Tyr Lys
70                  75                  80                  85

TGC TCG TAC CGG GAC GTC GAC ATA GCC TCC ACT GTT TAT GTC TAT GTT
Cys Ser Tyr Arg Asp Val Asp Ile Ala Ser Thr Val Tyr Val Tyr Val
                90                  95                  100

CGA GAT TAC AGA TCA CCA TTC ATC GCC TCT GTC AGT GAC CAG CAT GGC
Arg Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp Gln His Gly
            105                 110                 115

ATC GTG TAC ATC ACC GAG AAC AAG AAC AAA ACT GTG GTG ATC CCC TGC
Ile Val Tyr Ile Thr Glu Asn Lys Asn Lys Thr Val Val Ile Pro Cys
        120                 125                 130
```

Fig. 2.2

```
CGA GGG TCG ATT TCA AAC CTC AAT GTG TCT CTT TGC GCT AGG TAT CCA
Arg Gly Ser Ile Ser Asn Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro
    135                 140                 145

GAA AAG AGA TTT GTT CCG GAT GGA AAC AGA ATT TCC TGG GAC AGC GAG
Glu Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp Asp Ser Glu
150                 155                 160                 165

ATA GGC TTT ACT CTC CCC AGT TAC ATG ATC AGC TAT GCC GGC ATG GTC
Ile Gly Phe Thr Leu Pro Ser Tyr Met Ile Ser Tyr Ala Gly Met Val
                170                 175                 180

TTC TGT GAG GCA AAG ATC AAT GAT GAA ACC TAT CAG TCT ATC ATG TAC
Phe Cys Glu Ala Lys Ile Asn Asp Glu Thr Tyr Gln Ser Ile Met Tyr
            185                 190                 195

ATA GTT GTG GTT GTA GGA TAT AGG ATT TAT GAT GTG ATT CTG AGC CCC
Ile Val Val Val Val Gly Tyr Arg Ile Tyr Asp Val Ile Leu Ser Pro
        200                 205                 210

CCG CAT GAA ATT GAG CTA TCT GCC GGA GAA AAA CTT GTC TTA AAT TGT
Pro His Glu Ile Glu Leu Ser Ala Gly Glu Lys Leu Val Leu Asn Cys
    215                 220                 225

ACA GCG AGA ACA GAG CTC AAT GTG GGG CTT GAT TTC ACC TGG CAC TCT
Thr Ala Arg Thr Glu Leu Asn Val Gly Leu Asp Phe Thr Trp His Ser
230                 235                 240                 245

CCA CCT TCA AAG TCT CAT CAT AAG AAG ATT GTA AAC CGG GAT GTG AAA
Pro Pro Ser Lys Ser His His Lys Lys Ile Val Asn Arg Asp Val Lys
                250                 255                 260

CCC TTT CCT GGG ACT GTG GCG AAG ATG TTT TTG AGC ACC TTG ACA ATA
Pro Phe Pro Gly Thr Val Ala Lys Met Phe Leu Ser Thr Leu Thr Ile
            265                 270                 275

GAA AGT GTG ACC AAG AGT GAC CAA GGG GAA TAC ACC TGT GTA GCG TCC
Glu Ser Val Thr Lys Ser Asp Gln Gly Glu Tyr Thr Cys Val Ala Ser
        280                 285                 290

AGT GGA CGG ATG ATC AAG AGA AAT AGA ACA TTT GTC CGA GTT CAC ACA
Ser Gly Arg Met Ile Lys Arg Asn Arg Thr Phe Val Arg Val His Thr
    295                 300                 305

AAG CCT TTT ATT GCT TTC GGT AGT GGG ATG AAA TCT TTG GTG GAA GCC
Lys Pro Phe Ile Ala Phe Gly Ser Gly Met Lys Ser Leu Val Glu Ala
310                 315                 320                 325
```

Fig. 2.3

```
ACA GTG GGC AGT CAA GTC CGA ATC CCT GTG AAG TAT CTC AGT TAC CCA
Thr Val Gly Ser Gln Val Arg Ile Pro Val Lys Tyr Leu Ser Tyr Pro
                330                 335                 340

GCT CCT GAT ATC AAA TGG TAC AGA AAT GGA AGG CCC ATT GAG TCC AAC
Ala Pro Asp Ile Lys Trp Tyr Arg Asn Gly Arg Pro Ile Glu Ser Asn
            345                 350                 355

TAC ACA ATG ATT GTT GGC GAT GAA CTC ACC ATC ATG GAA GTG ACT GAA
Tyr Thr Met Ile Val Gly Asp Glu Leu Thr Ile Met Glu Val Thr Glu
        360                 365                 370

AGA GAT GCA GGA AAC TAC ACG GTC ATC CTC ACC AAC CCC ATT TCA ATG
Arg Asp Ala Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Met
    375                 380                 385

GAG AAA CAG AGC CAC ATG GTC TCT CTG GTT GTG AAT GTC CCA CCC CAG
Glu Lys Gln Ser His Met Val Ser Leu Val Val Asn Val Pro Pro Gln
390                 395                 400                 405

ATC GGT GAG AAA GCC TTG ATC TCG CCT ATG GAT TCC TAC CAG TAT GGG
Ile Gly Glu Lys Ala Leu Ile Ser Pro Met Asp Ser Tyr Gln Tyr Gly
                410                 415                 420

ACC ATG CAG ACA TTG ACA TGC ACA GTC TAC GCC AAC CCT CCC CTG CAC
Thr Met Gln Thr Leu Thr Cys Thr Val Tyr Ala Asn Pro Pro Leu His
            425                 430                 435

CAC ATC CAG TGG TAC TGG CAG CTA GAA GAA GCC TGC TCC TAC AGA CCC
His Ile Gln Trp Tyr Trp Gln Leu Glu Glu Ala Cys Ser Tyr Arg Pro
        440                 445                 450

GGC CAA ACA AGC CCG TAT GCT TGT AAA GAA TGG AGA CAC GTG GAG GAT
Gly Gln Thr Ser Pro Tyr Ala Cys Lys Glu Trp Arg His Val Glu Asp
    455                 460                 465

TTC CAG GGG GGA AAC AAG ATC GAA GTC ACC AAA AAC CAA TAT GCC CTG
Phe Gln Gly Gly Asn Lys Ile Glu Val Thr Lys Asn Gln Tyr Ala Leu
470                 475                 480                 485

ATT GAA GGA AAA AAC AAA ACT GTA AGT ACG CTG GTC ATC CAA GCT GCC
Ile Glu Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala
                490                 495                 500

AAC GTG TCA GCG TTG TAC AAA TGT GAA GCC ATC AAC AAA GCG GGA CGA
Asn Val Ser Ala Leu Tyr Lys Cys Glu Ala Ile Asn Lys Ala Gly Arg
            505                 510                 515
```

Fig. 2.4

```
GGA GAG AGG GTC ATC TCC TTC CAT GTG ATC AGG GGT CCT GAA ATT ACT
Gly Glu Arg Val Ile Ser Phe His Val Ile Arg Gly Pro Glu Ile Thr
        520                 525                 530

GTG CAA CCT GCT GCC CAG CCA ACT GAG CAG GAG AGT GTG TCC CTG TTG
Val Gln Pro Ala Ala Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Leu
    535                 540                 545

TGC ACT GCA GAC AGA AAT ACG TTT GAG AAC CTC ACG TGG TAC AAG CTT
Cys Thr Ala Asp Arg Asn Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu
550                 555                 560                 565

GGC TCA CAG GCA ACA TCG GTC CAC ATG GGC GAA TCA CTC ACA CCA GTT
Gly Ser Gln Ala Thr Ser Val His Met Gly Glu Ser Leu Thr Pro Val
                570                 575                 580

TGC AAG AAC TTG GAT GCT CTT TGG AAA CTG AAT GGC ACC ATG TTT TCT
Cys Lys Asn Leu Asp Ala Leu Trp Lys Leu Asn Gly Thr Met Phe Ser
            585                 590                 595

AAC AGC ACA AAT GAC ATC TTG ATT GTG GCA TTT CAG AAT GCC TCT CTG
Asn Ser Thr Asn Asp Ile Leu Ile Val Ala Phe Gln Asn Ala Ser Leu
        600                 605                 610

CAG GAC CAA GGC GAC TAT GTT TGC TCT GCT CAA GAT AAG AAG ACC AAG
Gln Asp Gln Gly Asp Tyr Val Cys Ser Ala Gln Asp Lys Lys Thr Lys
    615                 620                 625

AAA AGA CAT TGC CTG GTC AAA CAG CTC ATC ATC CTA GAG CGC ATG GCA
Lys Arg His Cys Leu Val Lys Gln Leu Ile Ile Leu Glu Arg Met Ala
630                 635                 640                 645

CCC ATG ATC ACC GGA AAT CTG GAG AAT CAG ACA ACA ACC ATT GGC GAG
Pro Met Ile Thr Gly Asn Leu Glu Asn Gln Thr Thr Thr Ile Gly Glu
                650                 655                 660

ACC ATT GAA GTG ACT TGC CCA GCA TCT GGA AAT CCT ACC CCA CAC ATT
Thr Ile Glu Val Thr Cys Pro Ala Ser Gly Asn Pro Thr Pro His Ile
            665                 670                 675

ACA TGG TTC AAA GAC AAC GAG ACC CTG GTA GAA GAT TCA GGC ATT GTA
Thr Trp Phe Lys Asp Asn Glu Thr Leu Val Glu Asp Ser Gly Ile Val
        680                 685                 690

CTG AGA GAT GGG AAC CGG AAC CTG ACT ATC CGC AGG GTG AGG AAG GAG
Leu Arg Asp Gly Asn Arg Asn Leu Thr Ile Arg Arg Val Arg Lys Glu
    695                 700                 705
```

Fig. 2.5

```
GAT GGA GGC CTC TAC ACC TGC CAG GCC TGC AAT GTC CTT GGC TGT GCA
Asp Gly Gly Leu Tyr Thr Cys Gln Ala Cys Asn Val Leu Gly Cys Ala
710                 715                 720                 725

AGA GCG GAG ACG CTC TTC ATA ATA GAA GGT GCC CAG GAA AAG ACC AAC
Arg Ala Glu Thr Leu Phe Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn
                730                 735                 740

TTG GAA GTC ATT ATC CTC GTC GGC ACT GCA GTG ATT GCC ATG TTC TTC
Leu Glu Val Ile Ile Leu Val Gly Thr Ala Val Ile Ala Met Phe Phe
            745                 750                 755

TGG CTC CTT CTT GTC ATT CTC GTA CGG ACC GTT AAG CGG GCC AAT GAA
Trp Leu Leu Leu Val Ile Leu Val Arg Thr Val Lys Arg Ala Asn Glu
        760                 765                 770

GGG GAA CTG AAG ACA GGC TAC TTG TCT ATT GTC ATG GAT CCA GAT GAA
Gly Glu Leu Lys Thr Gly Tyr Leu Ser Ile Val Met Asp Pro Asp Glu
    775                 780                 785

TTG CCC TTG GAT GAG CGC TGT GAA CGC TTG CCT TAT GAT GCC AGC AAG
Leu Pro Leu Asp Glu Arg Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys
790                 795                 800                 805

TGG GAA TTC CCC AGG GAC CGG CTG AAA CTA GGA AAA CCT CTT GGC CGC
Trp Glu Phe Pro Arg Asp Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg
                810                 815                 820

GGT GCC TTC GGC CAA GTG ATT GAG GCA GAC GCT TTT GGA ATT GAC AAG
Gly Ala Phe Gly Gln Val Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys
            825                 830                 835

ACA GCG ACT TGC AAA ACA GTA GCC GTC AAG ATG TTG AAA GAA GGA GCA
Thr Ala Thr Cys Lys Thr Val Ala Val Lys Met Leu Lys Glu Gly Ala
        840                 845                 850

ACA CAC AGC GAG CAT CGA GCC CTC ATG TCT GAA CTC AAG ATC CTC ATC
Thr His Ser Glu His Arg Ala Leu Met Ser Glu Leu Lys Ile Leu Ile
    855                 860                 865

CAC ATT GGT CAC CAT CTC AAT GTG GTG AAC CTC CTA GGC GCC TGC ACC
His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly Ala Cys Thr
870                 875                 880                 885

AAG CCG GGA GGG CCT CTC ATG GTG ATT GTG GAA TTC TCG AAG TTT GGA
Lys Pro Gly Gly Pro Leu Met Val Ile Val Glu Phe Ser Lys Phe Gly
                890                 895                 900
```

Fig. 2.6

```
AAC CTA TCA ACT TAC TTA CGG GGC AAG AGA AAT GAA TTT GTT CCC TAT
Asn Leu Ser Thr Tyr Leu Arg Gly Lys Arg Asn Glu Phe Val Pro Tyr
            905                 910                 915

AAG AGC AAA GGG GCA CGC TTC CGC CAG GGC AAG GAC TAC GTT GGG GAG
Lys Ser Lys Gly Ala Arg Phe Arg Gln Gly Lys Asp Tyr Val Gly Glu
        920                 925                 930

CTC TCC GTG GAT CTG AAA AGA CGC TTG GAC AGC ATC ACC AGC AGC CAG
Leu Ser Val Asp Leu Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln
    935                 940                 945

AGC TCT GCC AGC TCA GGC TTT GTT GAG GAG AAA TCG CTC AGT GAT GTA
Ser Ser Ala Ser Ser Gly Phe Val Glu Glu Lys Ser Leu Ser Asp Val
950                 955                 960                 965

GAG GAA GAA GAA GCT TCT GAA GAA CTG TAC AAG GAC TTC CTG ACC TTG
Glu Glu Glu Glu Ala Ser Glu Glu Leu Tyr Lys Asp Phe Leu Thr Leu
                970                 975                 980

GAG CAT CTC ATC TGT TAC AGC TTC CAA GTG GCT AAG GGC ATG GAG TTC
Glu His Leu Ile Cys Tyr Ser Phe Gln Val Ala Lys Gly Met Glu Phe
            985                 990                 995

TTG GCA TCA AGG AAG TGT ATC CAC AGG GAC CTG GCA GCA CGA AAC ATT
Leu Ala Ser Arg Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile
        1000                1005                1010

CTC CTA TCG GAG AAG AAT GTG GTT AAG ATC TGT GAC TTC GGC TTG GCC
Leu Leu Ser Glu Lys Asn Val Val Lys Ile Cys Asp Phe Gly Leu Ala
    1015                1020                1025

CGG GAC ATT TAT AAA GAC CCG GAT TAT GTC AGA AAA GGA GAT GCC CGA
Arg Asp Ile Tyr Lys Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg
1030                1035                1040                1045

CTC CCT TTG AAG TGG ATG GCC CCG GAA ACC ATT TTT GAC AGA GTA TAC
Leu Pro Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp Arg Val Tyr
                1050                1055                1060

ACA ATT CAG AGC GAT GTG TGG TCT TTC GGT GTG TTG CTC TGG GAA ATA
Thr Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile
            1065                1070                1075

TTT TCC TTA GGT GCC TCC CCA TAC CCT GGG GTC AAG ATT GAT GAA GAA
Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu
        1080                1085                1090
```

Fig. 2.7

TTT TGT AGG AGA TTG AAA GAA GGA ACT AGA ATG CGG GCT CCT GAC TAC
Phe Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro Asp Tyr
1095 1100 1105

ACT ACC CCA GAA ATG TAC CAG ACC ATG CTG GAC TGC TGG CAT GAG GAC
Thr Thr Pro Glu Met Tyr Gln Thr Met Leu Asp Cys Trp His Glu Asp
1110 1115 1120 1125

CCC AAC CAG AGA CCC TCG TTT TCA GAG TTG GTG GAG CAT TTG GGA AAC
Pro Asn Gln Arg Pro Ser Phe Ser Glu Leu Val Glu His Leu Gly Asn
1130 1135 1140

CTC CTG CAA GCA AAT GCG CAG CAG GAT GGC AAA GAC TAT ATT GTT CTT
Leu Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys Asp Tyr Ile Val Leu
1145 1150 1155

CCA ATG TCA GAG ACA CTG AGC ATG GAA GAG GAT TCT GGA CTC TCC CTG
Pro Met Ser Glu Thr Leu Ser Met Glu Glu Asp Ser Gly Leu Ser Leu
1160 1165 1170

CCT ACC TCA CCT GTT TCC TGT ATG GAG GAA GAG GAA GTG TGC GAC CCC
Pro Thr Ser Pro Val Ser Cys Met Glu Glu Glu Glu Val Cys Asp Pro
1175 1180 1185

AAA TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAT TAT CTC CAG AAC
Lys Phe His Tyr Asp Asn Thr Ala Gly Ile Ser His Tyr Leu Gln Asn
1190 1195 1200 1205

AGT AAG CGA AAG AGC CGG CCA GTG AGT GTA AAA ACA TTT GAA GAT ATC
Ser Lys Arg Lys Ser Arg Pro Val Ser Val Lys Thr Phe Glu Asp Ile
1210 1215 1220

CCA TTG GAG GAA CCA GAA GTA AAA GTG ATC CCA GAT GAC AGC CAG ACA
Pro Leu Glu Glu Pro Glu Val Lys Val Ile Pro Asp Asp Ser Gln Thr
1225 1230 1235

GAC AGT GGG ATG GTC CTT GCA TCA GAA GAG CTG AAA ACT CTG GAA GAC
Asp Ser Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp
1240 1245 1250

AGG AAC AAA TTA TCT CCA TCT TTT GGT GGA ATG ATG CCC AGT AAA AGC
Arg Asn Lys Leu Ser Pro Ser Phe Gly Gly Met Met Pro Ser Lys Ser
1255 1260 1265

AGG GAG TCT GTG GCC TCG GAA GGC TCC AAC CAG ACC AGT GGC TAC CAG
Arg Glu Ser Val Ala Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln
1270 1275 1280 1285

SUBSTITUTE SHEET (RULE 26)

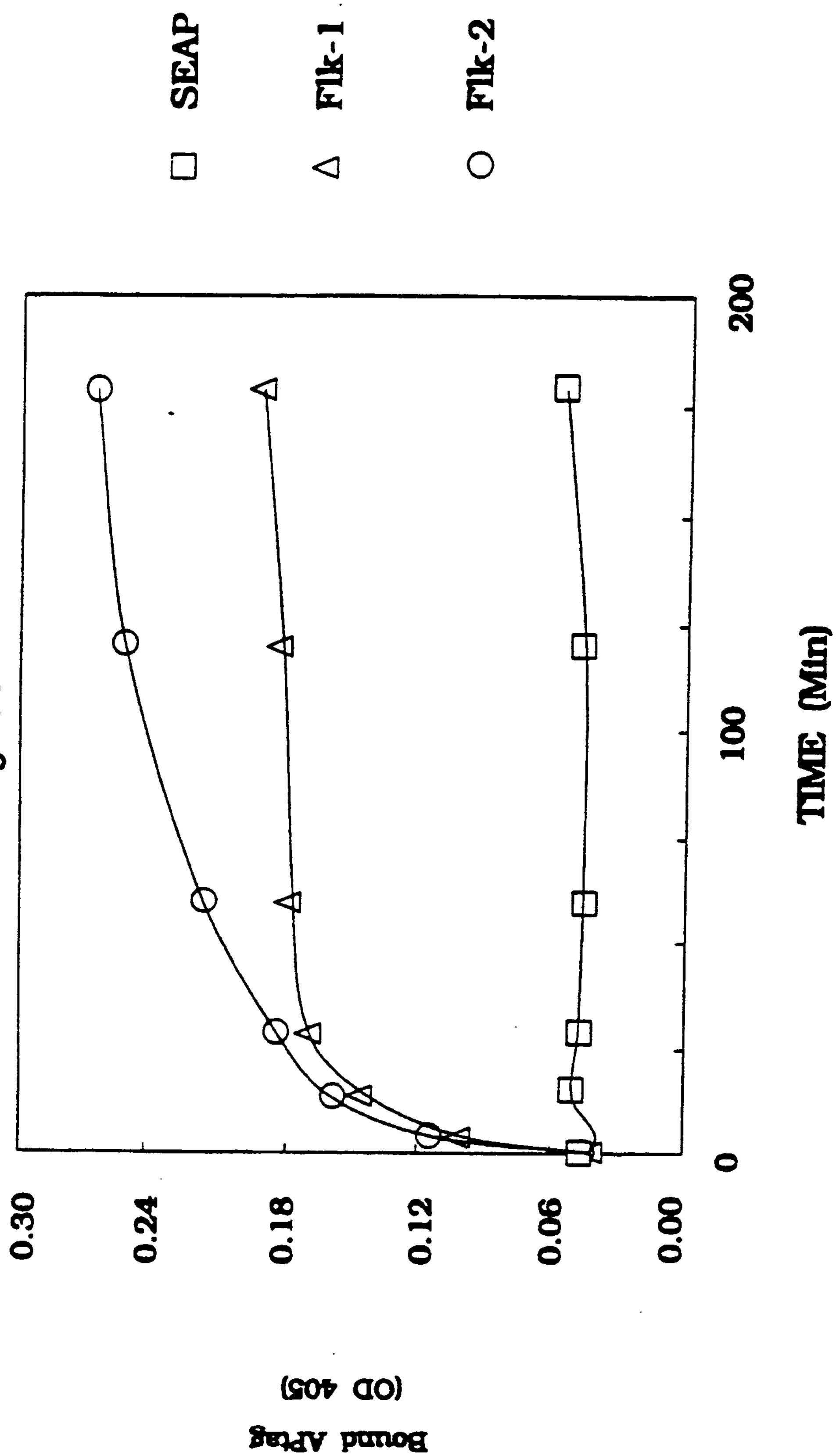
Fig. 2.8

```
TCT GGG TAT CAC TCA GAT GAC ACA GAC ACC ACC GTG TAC TCC AGC GAC
Ser Gly Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr Ser Ser Asp
                1290                1295                1300

GAG GCA GGA CTT TTA AAG ATG GTG GAT GCT GCA GTT CAC GCT GAC TCA
Glu Ala Gly Leu Leu Lys Met Val Asp Ala Ala Val His Ala Asp Ser
            1305                1310                1315

GGG ACC ACA CTG CAG CTC ACC TCC TGT TTA AAT GGA AGT GGT CCT GTC
Gly Thr Thr Leu Gln Leu Thr Ser Cys Leu Asn Gly Ser Gly Pro Val
        1320                1325                1330

CCG GCT CCG CCC CCA ACT CCT GGA AAT CAC GAG AGA GGT GCT GCT TAG
Pro Ala Pro Pro Pro Thr Pro Gly Asn His Glu Arg Gly Ala Ala
    1335                1340                1345

ATTTTCAAGT GTTGTTCTTT CCACCACCCG GAAGTAGCCA CATTTGATTT TCATTTTTGG
AGGAGGGACC TCAGACTGCA AGGAGCTTGT CCTCAGGGCA TTTCCAGAGA AGATGCCCAT
GACCCAAGAA TGTGTTGACT CTACTCTCTT TTCCATTCAT TTAAAAGTCC TATATAATGT
GCCCTGCTGT GGTCTCACTA CCAGTTAAAG CAAAAGACTT TCAAACACGT GGACTCTGTC
CTCCAAGAAG TGGCAACGGC ACCTCTGTGA AACTGGATCG AATGGGCAAT GCTTTGTGTG
TTGAGGATGG GTGAGATGTC CCAGGGCCGA GTCTGTCTAC CTTGGAGGCT TTGTGGAGGA
TGCGGCTATG AGCCAAGTGT TAAGTGTGGG ATGTGGACTG GGAGGAAGGA AGGCGCAAGC
CGTCCGGAGA GCGGTTGGAG CCTGCAGATG CATTGTGCTG GCTCTGGTGG AGGTGGGCTT
GTGGCCTGTC AGGAAACGCA AAGGCGGCCG GCAGGGTTTG GTTTTGGAAG GTTTGCGTGC
TCTTCACAGT CGGGTTACAG GCGAGTTCCC TGTGGCGTTT CCTACTCCTA ATGAGAGTTC
CTTCCGGACT CTTACGTGTC TCCTGGCCTG GCCCCAGGAA GGAAATGATG CAGCTTGCTC
CTTCCTCATC TCTCAGGCTG TGCCTTAATT CAGAACACCA AAAGAGAGGA ACGTCGGCAG
AGGCTCCTGA CGGGGCCGAA GAATTGTGAG AACAGAACAG AAACTCAGGG TTTCTGCTGG
GTGGAGACCC ACGTGGCGCC CTGGTGGCAG GTCTGAGGGT TCTCTGTCAA GTGGCGGTAA
AGGCTCAGGC TGGTGTTCTT CCTCTATCTC CACTCCTGTC AGGCCCCCAA GTCCTCAGTA
TTTTAGCTTT GTGGCTTCCT GATGGCAGAA AAATCTTAAT TGGTTGGTTT GCTCTCCAGA
```

Fig. 2.9

TAATCACTAG CCAGATTTCG AAATTACTTT TTAGCCGAGG TTATGATAAC ATCTACTGTA
TCCTTTAGAA TTTTAACCTA TAAAACTATG TCTACTGGTT TCTGCCTGTG TGCTTATGTT
AAAAAAAAAA AAAAA

Figure 3



SEAP
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Flk-2
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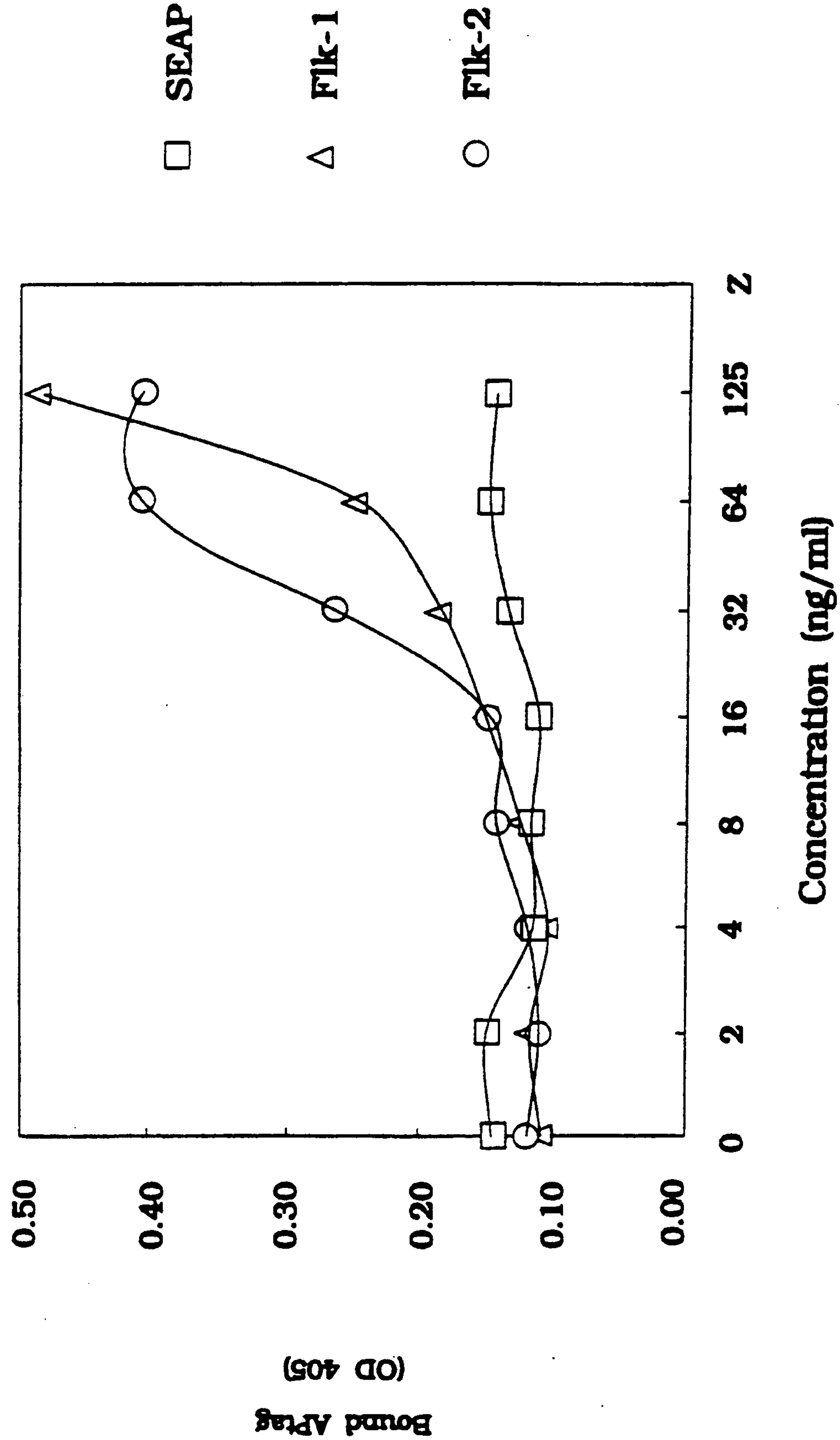
0.12
0.06
0.00
0
100
200
TIME (Min)
Bound APtag
(OD 405)

Figure 4



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0.30
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0.10
0.00
Bound APtag
(OD 405)
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2
4
8
16
32
64
125
Z
Concentration (ng/ml)